# A STUDY ON THE EFFECT OF GRK5 *Gln41Leu* POLYMORPHISM ON RESPONSE TO GLUCOCORTICOID THERAPY IN BRONCHIAL ASTHMA

**DISSERTATION**

**SUBMITTED FOR**

**M.D PHARMACOLOGY**

**THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY**


**DEPARTMENT OF PHARMACOLOGY**

**PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH**

**PEELAMEDU, COIMBATORE – 641004**

**TAMILNADU, INDIA**


**APRIL - 2016**

**PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH**

**COIMBATORE**

## CERTIFICATE

This is to certify that this dissertation entitled **"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma**" by **Dr.E.Amudhan Arvind**, is a work done by him during the period of study in the Department of Pharmacology from 2013 to 2016, under the guidance of **Dr.S.Ramalingam, M.D**., Professor, Department of Pharmacology and Dean, PSG IMS&R.

**Dr. K.Bhuvaneswari M.D**
Professor and Head,
Department of Pharmacology,
PSG IMS&R.

**Dr.S.Ramalingam M.D**
Professor and Guide
Department of Pharmacology,
PSG IMS&R.

**Dr.S.Ramalingam M.D.**

**DEAN**

**PSG IMS&R.**

## DECLARATION

I solemnly declare that the dissertation titled **"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma**" was done by me under the guidance and supervision of **Dr.S.Ramalingam M.D.,**

The dissertation is submitted to the Tamilnadu **Dr.M.G.R. Medical University** towards the partial fulfillment of the requirement for the award of **M.D. Degree in Pharmacology.**

Place :

Date : **Dr.E.AMUDHAN ARVIND**

## ACKNOWLEDGEMENT

I express my gratitude and sincere thanks to **Dr.S.Ramalingam M.D.,** Professor, Department of Pharmacology and Dean of PSG Institute of Medical Sciences & Research, for being my guide. It was his valuable suggestions, guidance and constant encouragement in every step that has helped me to complete my research work successfully. I am extremely indebted to him for allowing me to utilize the facilities of CMMT for my research work and for introducing me, the exciting field of molecular medicine.

I express my sincere thanks to **Dr.K.Bhuvaneshwari M.D.,** Professor and Head, Department of Pharmacology, PSGIMSR for her valuable support, suggestions and guidance.

A special thanks to **Dr. Dr.Sudha Ramalingam M.D.,** Assistant Professor, Department of Community Medicine, PSGIMSR for helping me to choosing this topic.

I am obliged to thank **Dr.RMPL.Ramanathan, Dr.Anupama Ramamurthy and Dr.Karthikeyan,** PSG Hospitals for their constant support in recruiting study participants.

I am thankful to **Dr.Thiyagarajan, Dr.Sudha Ramalingam, Mr.Sajeesh and Ms.Meenu** for their support and guidance in my genetic analysis.

I wish to express my whole hearted thanks to Professor **Dr.S.Bhuvaneshwari M.D.,** Professor **Dr.T.K.Ponnusamy M.D.,** Associate Professor **Dr.C.Deena Sangeetha M.D,**Assistant professors **Dr.S.Shanmugapriya M.D, Dr.N.Ramanujam M.D** and Senior resident Dr.G.Amudha for their advice and encouragement.

I wish to express my whole hearted thanks to my **Post Graduate Colleagues,** for giving me emotional support and for their timely help.

I also express my immense gratitude to the patients who consented to participate in this study.

I also thank my family members and especially my wife **Dr.Kalaiselvi** for the moral support they have rendered me throughout the study.

# PSG Institute of Medical Sciences & Research
## Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in


June 16, 2014

To
Dr E Amudhan Arvind
Postgraduate
Department of Pharmacology
PSG IMS & R
Coimbatore

**Ref.:** Proposal titled: *"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma"*

**Sub.:** Ethics Committee Approval for the study

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 10th June, 2014 in its full board review meeting held at Research Conference Room, PSG IMS&R, between 9.30 am and 12.30 pm, and discussed your application to conduct the study entitled:

*"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma"*

The following documents were received for review:

1. Duly filled application form
2. Proposal
3. Informed Consent forms
4. Data Collection Tool
5. Permission letter from concerned Heads of Department
6. CV
7. Budget

The members who attended the meeting at which your study proposal was discussed are as follows:

| Sl. No. | Name of the Member of IHEC | Qualification | Area of Expertise | Gender | Affiliation to the Institution Yes/No | Present at the meeting Yes/No |
|---|---|---|---|---|---|---|
| 1 | Dr. S. Bhuvaneshwari (Member-Secretary, IHEC) | MD | Clinical Pharmacology | Female | Yes | Yes |
| 2 | Mrs. Geetha S Kannan | + 2 | Lay person | Female | No | Yes |
| 3 | Mr Gowpathy Velappan | BA., BL | Legal Advisor | Male | No | Yes |
| 4 | Mrs G Malarvizhi | M Sc | Nursing | Female | Yes | No |
| 5 | Mr. R. Nandakumar (Vice-Chairperson, IHEC) | BA., BL | Legal Expert | Male | No | Yes |
| 6 | Dr. G. Rajendiran | DM | Clinician (Cardiology) | Male | Yes | Yes |
| 7 | Dr. V. Ramamurthy | Ph D | Biotechnology | Male | Yes | No |

# PSG Institute of Medical Sciences & Research
## Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in


| | | | | | | |
|---|---|---|---|---|---|---|
| 8 | Dr. M. Ramanathan | M Pharm, Ph D | Non-Medical (Pharmacy) | Male | Yes | Yes |
| 9 | Dr. P. Sathyan (Chairperson, IHEC) | DO, DNB | Clinician (Opthalmology) | Male | No | Yes |
| 10 | Dr. Seetha Panicker | MD | Clinician (Obstetrics & Gyanaecology) | Female | Yes | No |
| 11 | Dr. S. Shanthakumari | MD | Pathology, Ethicist | Female | Yes | Yes |
| 12 | Dr. Y.S. Sivan | Ph D | Social Scientist (Sociology) | Male | Yes | Yes |
| 13 | Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC) | MD | Public Health, Epidemiology, Genetics, Ethicist | Female | Yes | Yes |
| 14 | Mrs. K. Uma Maheswari | M Sc, M Phil. B Ed | Botany | Female | No | No |
| 15 | Dr. D. Vijaya | M Sc, Ph D | Basic Medical Sciences (Biochemistry) | Female | Yes | Yes |

After due consideration, the committee has decided to approve the above proposal.

The approval is valid for one year.

**We request you to intimate the date of initiation of the study to IHEC, PSG IMS&R and also, after completion of the project, please submit completion report to IHEC.**

We hereby confirm that neither you nor any of your study team members have participated in the voting/ decision making procedure of the committee. The members of the committee who have participated in the voting/ decision making procedure of the committee do not have any conflict of interest in the referenced study.

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

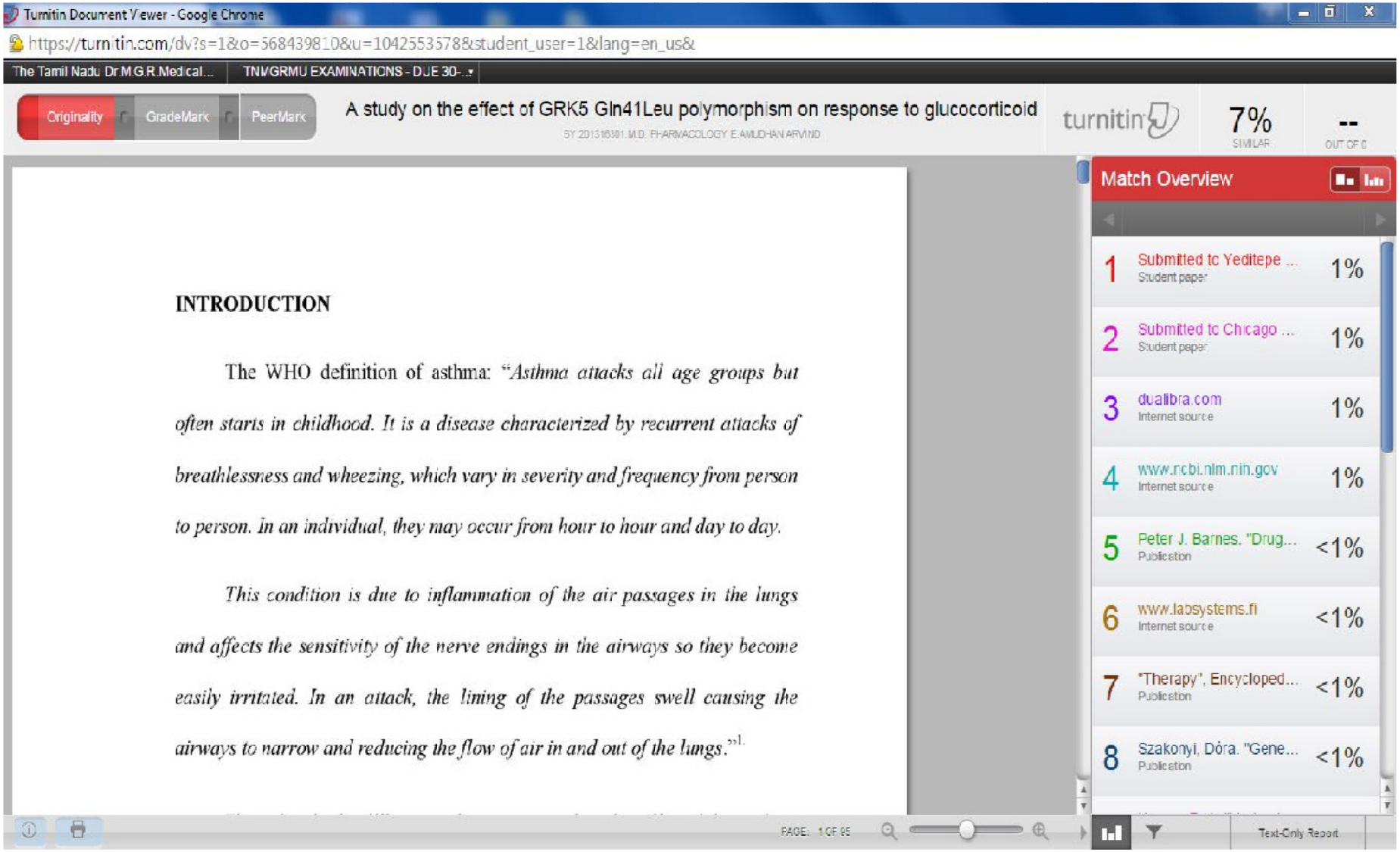
PIs are required to send progress reports (in the form of an extended abstract with publications if any) to the IHEC every six months (and a month before expiry of approval date, if renewal of approval is being sought).

Request for renewal must be made at least a month ahead of the expiry of validity along with a copy of the progress report.

16.6.17

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

**Dr S Bhuvaneshwari**
**Member - Secretary**
**Institutional Human Ethics Committee**

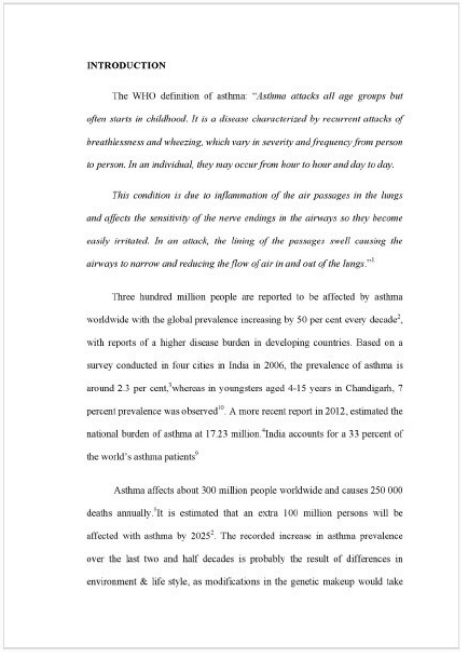

Turnitin Document Viewer - Google Chrome
https://turnitin.com/dv?s=1&o=568439810&u=1042553578&student_user=1&lang=en_us&
The Tamil Nadu Dr.M.G.R.Medical...
TNMGRMU EXAMINATIONS - DJE 30-...
Originality
GradeMark
PeerMark
A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid
turnitin
7%
SIMILAR
--
OUT OF 0
INTRODUCTION
The WHO definition of asthma: "Asthma attacks all age groups but often starts in childhood. It is a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. In an individual, they may occur from hour to hour and day to day.
This condition is due to inflammation of the air passages in the lungs and affects the sensitivity of the nerve endings in the airways so they become easily irritated. In an attack, the lining of the passages swell causing the airways to narrow and reducing the flow of air in and out of the lungs."[1].
Match Overview
1 Submitted to Yeditepe ... Student paper 1%
2 Submitted to Chicago ... Student paper 1%
3 dualibra.com Internet source 1%
4 www.ncbi.nlm.nih.gov Internet source 1%
5 Peter J. Barnes. "Drug... Publication <1%
6 www.labsystems.fi Internet source <1%
7 "Therapy", Encycloped... Publication <1%
8 Szakonyi, Dóra. "Gene... Publication <1%
Text-Only Report

# Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

| | |
|---|---|
| Submission author: | 201316301.m.d. Pharmacology E.AM. |
| Assignment title: | TNMGRMU EXAMINATIONS |
| Submission title: | A study on the effect of GRK5 Gln... |
| File name: | Amudhan_Dissertation_final_form... |
| File size: | 1.82M |
| Page count: | 95 |
| Word count: | 12,126 |
| Character count: | 68,668 |
| Submission date: | 26-Sep-2015 09:05AM |
| Submission ID: | 568439810 |



**INTRODUCTION**

The WHO definition of asthma: "*Asthma attacks all age groups but often starts in childhood. It is a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. In an individual, they may occur from hour to hour and day to day.*

*This condition is due to inflammation of the air passages in the lungs and affects the sensitivity of the nerve endings in the airways so they become easily irritated. In an attack, the lining of the passages swell causing the airways to narrow and reducing the flow of air in and out of the lungs.*"[1]

Three hundred million people are reported to be affected by asthma worldwide with the global prevalence increasing by 50 per cent every decade[2], with reports of a higher disease burden in developing countries. Based on a survey conducted in four cities in India in 2006, the prevalence of asthma is around 2.3 per cent,[3]whereas in youngsters aged 4-15 years in Chandigarh, 7 percent prevalence was observed[10]. A more recent report in 2012, estimated the national burden of asthma at 17.23 million.[4]India accounts for a 33 percent of the world's asthma patients[5]

Asthma affects about 300 million people worldwide and causes 250 000 deaths annually.[7]It is estimated that an extra 100 million persons will be affected with asthma by 2025[2]. The recorded increase in asthma prevalence over the last two and half decades is probably the result of differences in environment & life style, as modifications in the genetic makeup would take

# CONTENTS

# ABSTRACT

## Introduction

Asthma is a chronic inflammatory disease characterized by hyper-reactive airways causing recurrent episodes of wheezing, breathlessness and coughing, particularly at night or early in the morning. It has a familial preponderance with a polygenic inheritance. Various genetic alterations, single nucleotide polymorphisms (SNPs), are known to affect the effective management of asthma. One such polymorphism that has not been effectively explored is the G protein coupled receptor kinase5 (GRK5).This study aims to explore the effect of GRK5 polymorphism on the control and management of bronchial asthma in patients who are receiving inhaled corticosteroids.

## Objectives & Methodology

Our objectives were to study the influence of GRK5 polymorphism on the dose and duration of treatment with inhaled glucocorticoids in asthmatics, and to analyze the effect of GRK5 polymorphism on disease control. After IHEC approval, we obtained written informed consent from asthmatics receiving inhaled steroids, collected 2 ml of blood and did a genetic analysis for GRK5 polymorphism on the sample. We also collected details of drugs received, dose and duration of therapy. Patients were classified as those requiring high dose or low dose inhaled steroid based on the GINA Guidelines for Inhaled steroids Dose Equivalence Table. The duration of treatment was classified into long term (>3 months) or short term (<3 months). We analysed the results from collected data.

## Results & Discussion

Patients with GRK5 polymorphism required high dose inhaled steroids. This association was significant ($p<0.005$) with an odds ratio of 26.133. However, the polymorphism did not have any significant effect on the duration of treatment. Surprisingly, patients with GRK5 polymorphism had good disease control ($p=0.004$) with an odds ratio of 6.283. This is in contrast to the expectation that patients with the SNP would have poor disease control due to endogenous beta blockade. This finding can be explained by the fact that most patients with GRK5 polymorphism required high dose inhaled steroid, which may have attenuated disease severity. We also analysed all the co-factors which could influence disease control, dose and duration of steroid use, using multivariate regression model which confirmed the above results.

## Conclusion:

Asthmatics with GRK5 polymorphism have a higher probability of requiring high dose steroids for adequate control of disease. GRK5 polymorphism does not have a significant effect on the duration of drug therapy. Though patients with polymorphism are expected to have poor control of their asthma, intake of high dose steroids may reverse the effect of the polymorphism and these patients may actually have adequate control of their disease. This study is the first of its type done in India - analysing the effect of GRK5 polymorphism on steroid therapy in asthmatics.

**Keywords:** Bronchial asthma, GRK5 Polymorphism,Dose,Duration,Control,Inhaled steroids

## INTRODUCTION

*World Health Organisation defines asthma as a disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person. In an individual, they may occur from hour to hour and day to day.*

*This condition is due to inflammation of the air passages in the lungs and affects the sensitivity of the nerve endings in the airways so they become easily irritated. In an attack, the lining of the passages swell causing the airways to narrow and reducing the flow of air in and out of the lungs*."[1.]

Three hundred million people are reported to be affected by asthma worldwide with the global prevalence increasing by 50 per cent every decade[2], with reports of a higher disease burden in developing countries. Based on a survey conducted in four cities in India in 2006, the prevalence of asthma is around 2.3 per cent,[3]whereas in youngsters aged 4-15 years in Chandigarh, 7 percent prevalence was observed[10]. A more recent report in 2012, estimated the national burden of asthma at 17.23 million.[4]India accounts for a 33 percent of the world's asthma patients[9]

Asthma affects about 300 million people worldwide and causes 250 000 deaths annually.[5]It is estimated that an extra 100 million persons will be affected with asthma by 2025[2]. The recorded increase in asthma prevalence over the last two and half decades is probably the result of differences in environment & life style, as modifications in the genetic makeup would take

many generations to occur[6]. Worldwide, the number of cases is doubling every ten years &the WHO expects asthma, beside COPD, to become the 3rdmaincause of mortality by 2020.

A multicentric epidemiological study on the prevalence of asthma among Indian adults using a uniform, valid and standardized methodology showed that higher age groups, females, family history of respiratory diseases, known history of atopy or allergy, lower socio-economic status and urbanization were related to asthma[7]. In another study in Delhi, parental smoking, NSAID usage, exposure to pets, pollution due to traffic congestion were shown to be associated with wheezing[8].

Multiple studies have been carried out to investigate the causes and risk factors for developing asthma. It has been studied and confirmed that various genes and factors in the environment increase the susceptibility to disease[11]. There is evidence of familial aggregation of asthma and atopy, and having a family history of asthma is considered to be one of the most important risk factors for developing the disease[12]

Globally, the cost related to respiratory problems exceeds that of TB and HIV/AIDS combined[13]. Developed countries expect to spend 1-2% of their health budget on Asthma[2].Thus the burden of the disease is not just the associated morbidity and mortality, but also the weakened economic status of the patient. Thus there is a need for aggressive management.

Despite the presence of highly effective curative treatment modalities for

the treatment of asthma, the disease is often inadequately controlled in many patients.

Several reasons are quoted for this poor management: patient non-compliance to treatment and follow up, poor inhalation technique[14], presence of co-morbid diseases[15], triggers like gastro-esophageal reflux disease,[16] respiratory infections[17], indoor allergens[18], risky environmental exposures[19]and lack of response to medications[20].

Most asthmatics are on chronic treatment with inhaled corticosteroids and their disease control depends on their adherence to treatment.Many genetic factors can also influence the prevalence and control of asthma, in addition to the environmental and biological factors. Various genetic alterations, single nucleotide polymorphisms (SNPs) are known to affect the effective management of asthma: ADRB2 gene, IL 17, TNF alpha, ADAM 33, DPP10, GRPA, etc[21].

One such polymorphism that has not been sufficiently explored is the G protein coupled receptor kinase5 (GRK5).This study aims to explore the effect of GRK5 polymorphism on the control and management of bronchial asthma in patients who are receiving inhaled corticosteroids.

# *Aims & Objectives*

**OBJECTIVES:**

**Primary Objective:**

1. To study the effect of GRK5 polymorphism on the usage of glucocorticoids in patients of bronchial asthma

**Secondary Objectives**

1. To study the influence of polymorphism on dosage and duration of treatment with inhaled glucocorticoids.
2. To analyze the effect of GRK5 polymorphism on disease control of asthma in patients on glucocorticoids.

# REVIEW OF LITERATURE

## DEFINITION OF ASTHMA

The word "asthma" is a Greek word which means "panting" or "gasping for breath." Asthma has been known to mankind since ancient times.

The concepts of this disease have changed from one hypothesis to another. In the 19th century, Henry Hyde Salter[22]proposed that both neural and vascular mechanisms were involved in disease development. He stated that "*The inflammation or congestion of mucous surface appears to be the stimulus that, through the nerves of the air tubes, excites the muscular wall to contract*". Many authors of that time agree that there was a strong neurotic element in cases of asthma. Thus, asthma was largely considered to be a psychoneurosis.

However research done in the late 19th century tilted the etiology towards various environmental factors. Pollen was demonstrated to be the cause of hay asthma by Charles Blackle[23].Similarly, the confirmation of smooth muscle antigenic sensitizations in animal models of disease[24] and the discovery of sensitizing proteins[25] further strengthened the role of environmental factors in chronic asthmatics. Rackemann[26] further differentiated asthma as intrinsic and extrinsic based on environmental triggers.

The CIBA foundation guest symposium[27] held in 1958, attempted to define asthma: "*Asthma refers to a condition in subjects with widespread narrowing of bronchial airways which changes in severity over short periods*

*of time either spontaneously or under treatment and is not due to cardiovascular disease*"[27].

In 1990, in view of the high causalities' due to asthma in England, the British Thoracic Society released guidelines for the management of asthma[28]. As per these guidelines, "*Asthma is a common and chronic inflammatory condition of the airways whose cause is not completely understood. As a result of inflammation the airways are hyper-responsive and they narrow easily in response to a wide range of stimuli. This may result in symptoms like coughing, wheezing, chest tightness and dyspnea; these symptoms are often worse at night. Narrowing of the airway is usually reversible, but in some patients with chronic asthma, the inflammation may lead to irreversible obstruction of airflow resulting in poor control*"[28].

However it took nearly a century to reach a consensus. In 1991, the National Heart Lung & Blood Institute (NHLBI) formulated an expert panel group[29]which put forth a guide for the definition and management of asthma: "*Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role: In particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheeze, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment .The inflammation also causes an associated increase in the*

*existing bronchial hyper-responsiveness to a variety of stimuli. Reversibility of airflow limitation may be incomplete in some patients with asthma*."[29, 30]

Finally as per the current Global Initiative for Asthma (GINA) guidelines[31] "*Asthma is a chronic inflammatory disorder of the airways in which many cellular events play a role. This chronic inflammation is associated with airway hyper-responsiveness that leads to recurrent episodes of wheezing, breathlessness chest tightness and coughing, particularly at night or early in the morning. These episodes are usually associated with airflow obstruction within the lung that is often reversible either spontaneously or with treatment*."

## PATHOPHYSIOLOGY

Asthma is associated with chronic inflammation of the mucous membrane of the lower respiratory tract. Thus reduction of inflammation is one of the main targets of management.

## PATHOLOGY

The airway mucous membrane is infiltrated with mast cells, active eosinophils and T lymphocytes. This inflammation is usually reduced by treatment with corticosteroids. Un-treated and under-treated disease results in remodeling of the airways. Basement membrane thickening as a result of collagen deposition in the sub epithelial region is observed. The epithelial tissue is often weak and has decreased fixation to the bronchial wall, with a higher number of epithelial cells within the airway duct. Other features include

edematous and thick airways accompanied by obstruction of bronchi due to formation of plugs by mucous. Vasodilatation and angiogenesis also occur.

When viewed with a bronchoscope, the large airways may be narrow, erythematous and edematous. Peripheral inflamed airways are found mainly in severe asthma with uneven narrowing of airways.

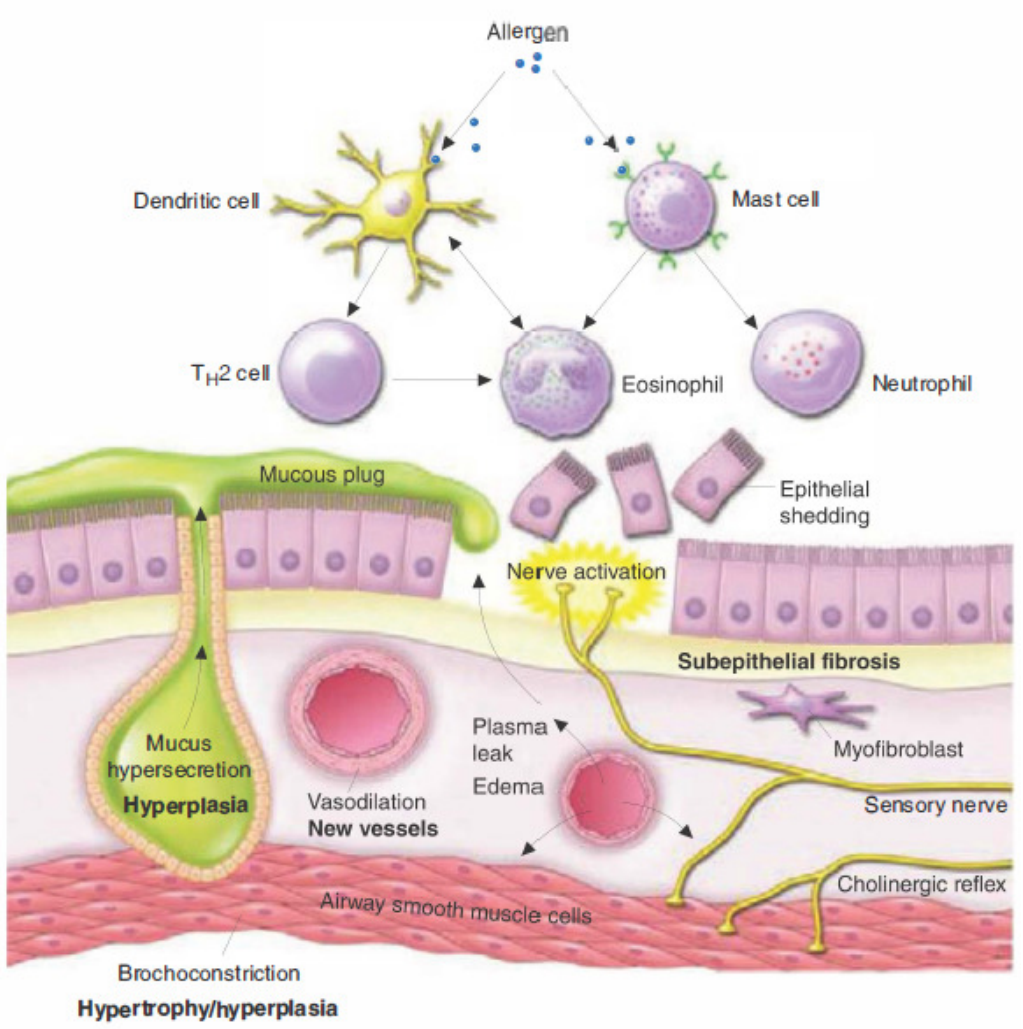
- **Airway Inflammation**

Inflammation is seen from the trachea to the terminal bronchioles. There is good evidence of association between the pattern of inflammation and hyper responsiveness with variable airflow obstruction[32]**.**The inflammation in intrinsic asthma is unique that it is dependent on local IgE production. Acute inflammatory episodes are superimposed on this chronic inflammatory state which causes acute exacerbations. Although eosinophil infiltration is common, some patients with severe asthma have a neutrophilic pattern of inflammation that is less sensitive to corticosteroids.

- **Mast cells**

They initiate response to allergens & other stimuli, like exercise and hyperventilation. Activated cells are found at the bronchial epithelium& also in the airway smooth-muscle layer but are normally absent in eosinophilic bronchitis. Allergens stimulate mast cells through IgE[33]. They also release several mediators of bronchoconstriction.

**Image1: Pathophysiology of Asthma**




- **Macrophages and dendritic cells**

Macrophages move within the lumen in asthmatics and get activated by allergens. Dendritic cells in the airway epithelium take up allergens and planthe assembly of T cells specific to allergens [34].

- **Eosinophils**

Eosinophil infiltration is a typical facet of asthma and is linked tothe development of airway hyper-responsiveness (AHR). They are important in airway remodeling and in exacerbations. Reduction of the activity of IL-5 causes reduction in the circulating eosinophils[35].

- **Neutrophils**

Severe asthmatics have more activated neutrophils in phlegm and the airway. Few patients with mild or moderate disease also have higher numbers of airway neutrophils[36].

- **Structural cells**

Structural cells are main sources of release of provocative intermediaries. Epithelial cells have important roles in transporting inhaled stimulants into an airway and are most likely important target cells meant for inhaled corticosteroids (ICS) [39].

- **T lymphocytes**

They have a significant role in coordinating the inflammatory response in asthma by releasing various cytokines. In asthmatics, TH2 cells predominate in the airways, while there are more TH1 cells in normal persons. TH2 cells cause eosinophilic inflammation and also increase IgE formation[37].

- **Inflammatory Mediators**

Multiple inflammatory mediators are involved in asthma and have different effects on airways causing various pathophysiologic changes.[40]

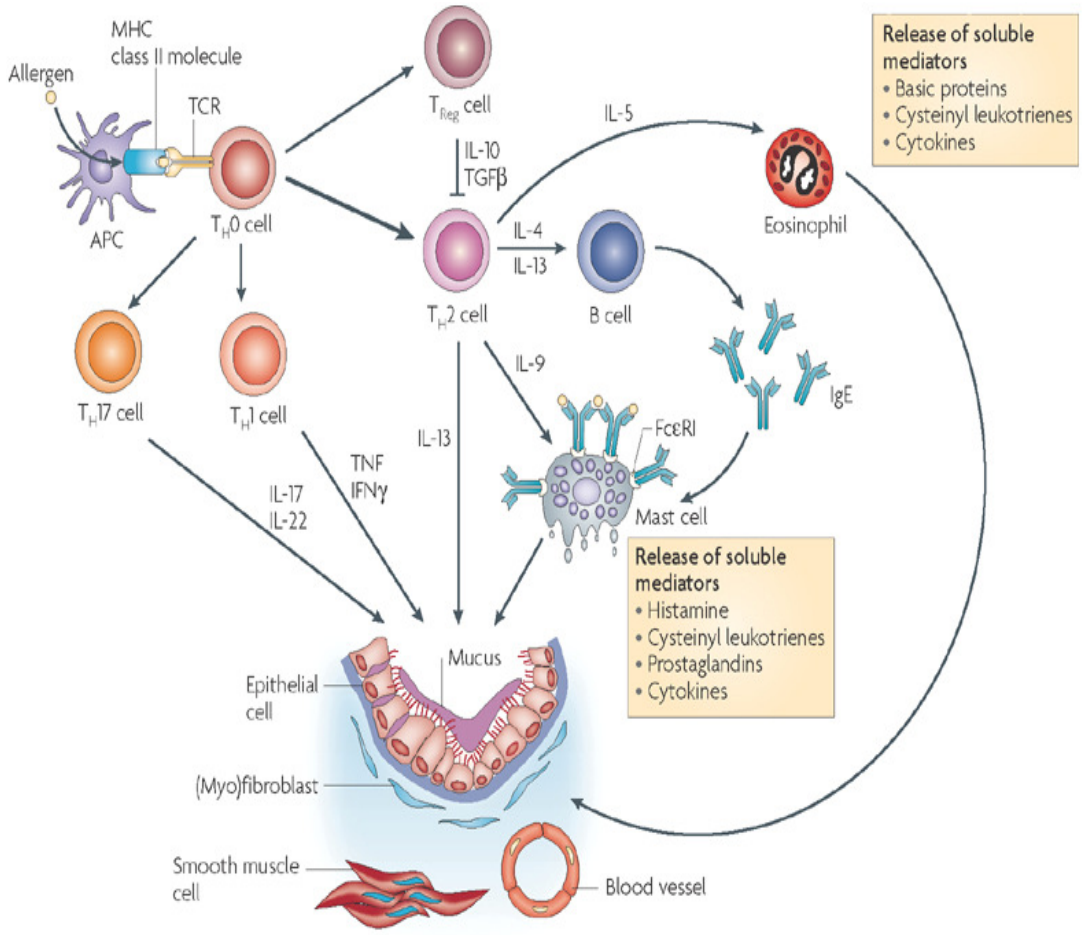
**a) Cytokines**

Role of cytokines in asthma:

- TH2 cells, cytokines IL-4, IL-5, and IL-1 →allergic inflammation
- TNF-α,IL-1β → amplify the inflammatory response
- Thymic stromal lymphopoietin(TSLP) →release of chemokines that selectively attract TH2 cells

- IL-10,IL-12 are anti-inflammatory and may be deficient in asthma [41]

**Image 2: Effect of T Lymphocytes on asthma during inflammation**[38]

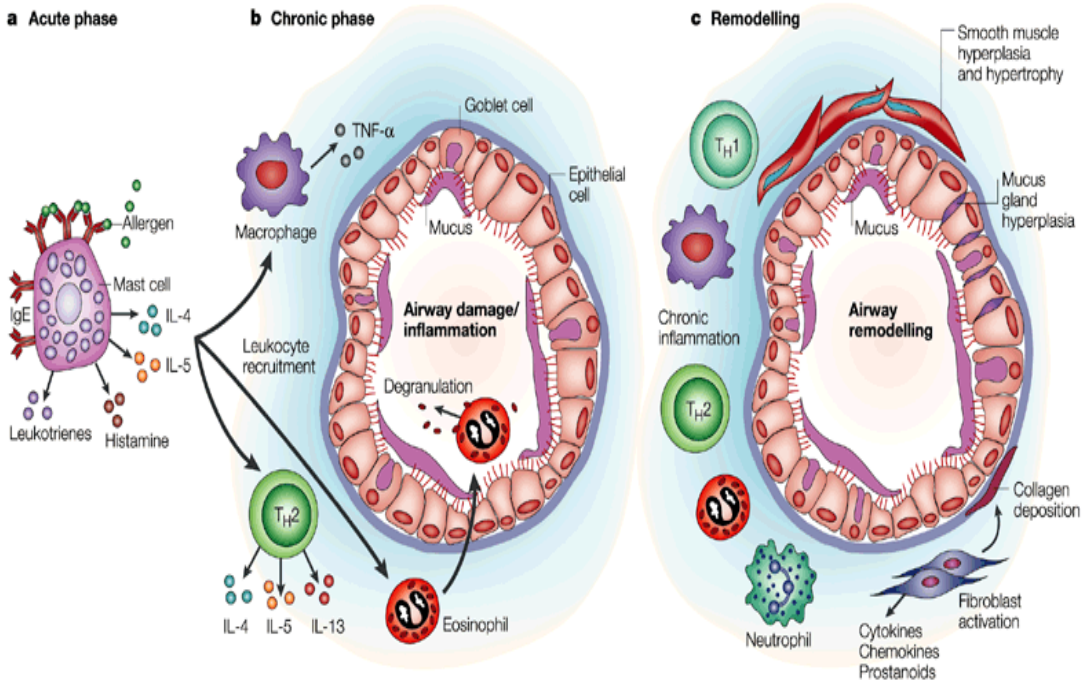



**b) Chemokines**

They act by drawing inflammatory mediators towards the airways from the circulation[42, 43].

- CCL17 (TARC) & CCL22 (MDC)

**Image 3: Inflammatory mediators in asthma[146]**




- **Oxidative Stress**

Activated inflammatory cells produce reactive oxygen species (ROS). Augmented oxidative stress is indicated by increased concentration of 8-isoprostane and ethane in these patients[44]. Increased oxidative stress reduces the response to corticosteroids[45].

- **Nitric oxide**

Nitric oxide (NO) is formed in the airway epithelial cells and macrophages[46]. Higher level of NO is exhaled in asthmatics and may be the cause of bronchial vasodilatation in asthma.

## EFFECTS OF CHRONIC INFLAMMATION ON THE AIRWAYS

- **Airway epithelium**

Bronchial epithelial shedding is causative of AHR in many ways: decrease in barrier function, loss of enzymes that degrade certain peptide inflammatory mediators,[48]loss of endothelium derived relaxing factor (EDRF) and reflex neural effects on the airway.

- **Fibrosis**

There will be sub-epithelial fibrosis with eosinophilic infiltration through release of TGF-β.This causes narrowing of the airways leading to poor control[49, 50].

- **Airway smooth muscle**

Inflammatory mediator's alter ionic channels that control the resting membrane potential of airway smooth-muscle cells, hence altering their excitability. The hypertrophy and hyperplasia[51] seen in airway smooth muscle in asthmatic bronchi is due to factors like platelet-derived growth factor (PDGF) or EDRF.

- **Vascular responses**

A rise in bronchial mucosal blood flow contributes to airway narrowing. There is angiogenesis in asthmatic airways in response to growth factors particularly vascular endothelial growth factor (VEGF)[52].Microvascular leakage from post capillary venules causes edema and plasma exudation.

- **Mucus hyper-secretion**

Augmented mucus exudation causes sticky plugs that obstruct diseased airways. There will be gland hyperplasia in the sub-mucosal region of larger airways& increase in epithelial goblet cells[53].

- **Neural regulation**

Abnormalities in the autonomic pathway add to AHR through discharge of acetylcholine (Ach)which acts on muscarinic receptors, causing bronchial constriction. Mediators cause reflex cholinergic bronchoconstriction and release of neuropeptides[54].

- **Airway Remodeling**

As per studies there will be a decline in lung function due to airway remodeling; if treated properly patients can maintain normal or near normal lung function. Only severe asthmatics show decline in function even after treatment[55].

- **Airway Hyper-responsiveness**

AHR is the distinctive feature of asthma which is nothing but excessive bronchoconstrictor response to multiple triggers. Rise in AHR is proportional to the occurrence of symptoms, and the main focus of treatment is to minimize AHR.[56]

## RISK FACTORS:

Many environmental and genetic risk factors can provoke asthmatic symptoms.

### 1) Atopy

Atopics have a high probability of becoming asthmatics. Asthmatics often suffer from allergic rhinitis, which suggests that both genetic and environmental factors influence development of disease in atopic individuals[57].

### 2) Genetic Predisposition

The familial association of asthma points to a genetic predisposition of asthma. There are many new studies which indicate that the severity depends on genotype. Molecular screening with linkage analysis and SNP's of various genes indicates that asthma has a polygenic inheritance[58].Newer genes have been related to asthma, like *ADAM-33*,*GRK5* and *DPP-10*, but their effect in disease pathogenesis is not yet clear[59]. Genetic polymorphisms may also be significant in shaping the reaction to disease management.

### GPCR & GRK 5

G protein coupled receptors (GPCRs) sense a wide range of extracellular signals, like

- Hormones
- Chemokines
- Light
- Odorants and Neurotransmitters

Over 1000 types of this receptor are present. All GPCRs known so far mediate signals from extracellular to intracellular by stimulating G proteins[60].

GPCRs span around the plasma membrane in a bundle of 7 α helices[61]. Humans express over 800 GPCRs which forms the 3rd biggest group of genes, with half of these GPCRs governing sensory perception (smell, taste, and vision). The remaining receptors regulate many physiological functions including nerve activity, tension of smooth muscle, metabolism, rate and force of cardiac contraction, and the secretion of most glands in the body. Some of the ligands for GPCRs are

- Eicosanoids
- Peptide hormones
- Neurotransmitters - Acetyl choline
- Lipid signaling molecules
- Opioids
- Biogenic amines - Nor epinephrine
- Amino acids – GABA

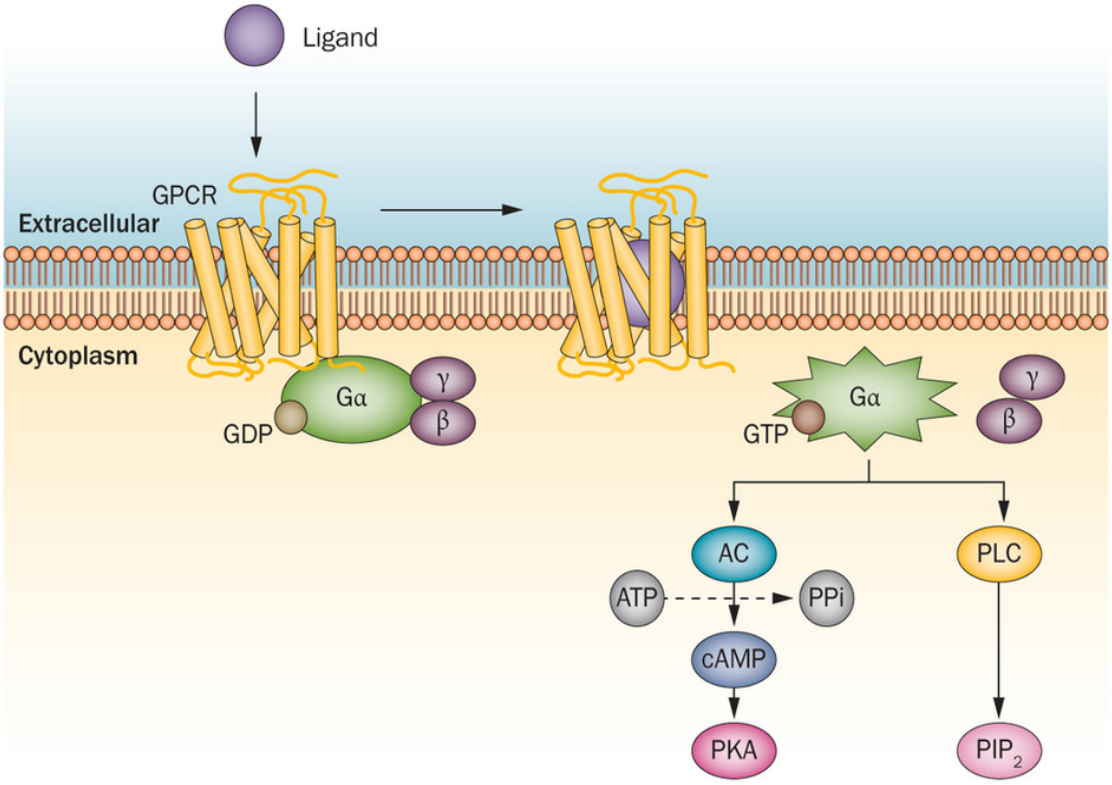
GPCRs are important regulators of nerve activity in the central nervous system (CNS) and are the receptors for the neurotransmitters of the peripheral autonomic nervous system (ANS). Due to their number, physiological importance, GPCRs are targets for many drugs; *almost half of non-antibiotic drugs act through these receptors.*

## ACTIVATION

When an agonist binds to a GPCR, there is a conformational change in the receptor that is transmitted from the ligand-binding pocket to the second and third intracellular loops of the receptor which couple to the G protein heterotrimer. This conformational change causes α subunit to exchange its bound GDP for GTP. Binding of GTP activates the α subunit and causes it to release both the βγ dimer and the receptor, and both the GTP-bound α subunit and the βγ heterodimer become active signaling molecules[62]. The interaction of the agonist-bound GPCR with G protein is temporary; following activation of one G protein, the receptor is freed to interact with other G proteins. Depending on the nature of the α subunit, the active, GTP-bound form binds to and regulates effectors such as adenylyl cyclase (via $G_s\alpha$) or phospholipase C (via $G_q\alpha$).

The βγ subunit can regulate many effectors including ion channels and enzymes such as $PI_3$-K. The G protein is dynamic until GTP bound to alpha subunit is hydrolyzed to GDP. The alpha subunit has a slow intrinsic rate of GTP hydrolysis that is modified by *regulators of G protein signaling* (RGSs). These proteins greatly accelerate hydrolysis of GTP and are potentially attractive drug targets[63].

**Image 4: Activation of GPCR[147]**




**DESENSITISATION**

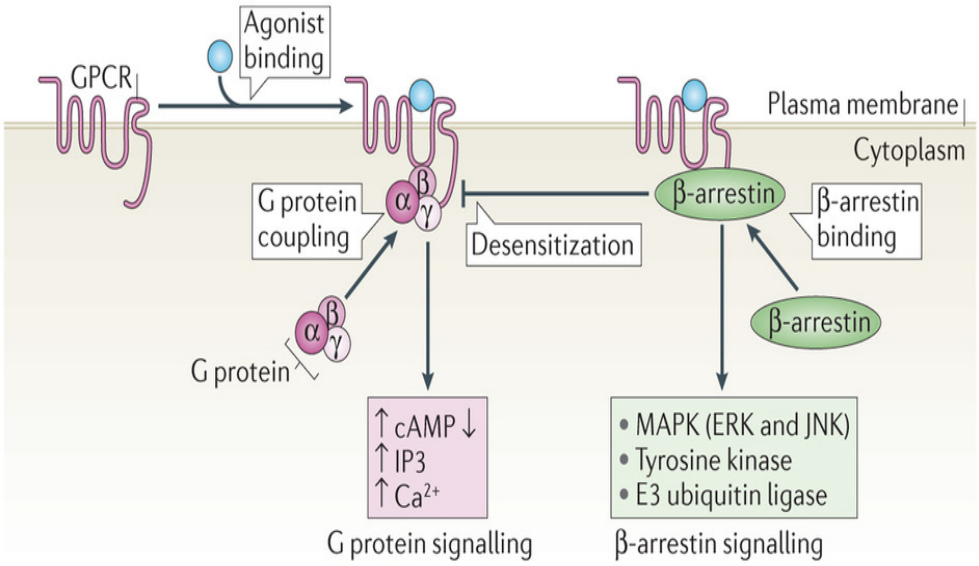
Receptors in addition to regulation of biochemical process & physiological function also subject themselves to many homeostatic and regulatory controls. These controls include

- Covalent modification
- Regulation of synthesis
- Association with other regulatory proteins
- Degradation of receptor and
- Relocalization within cell.

Similar regulation of transducer and effector protein occurs through modulatory inputs from other receptors, and there is also self-regulation by feedback regulation of their own signaling outputs.

Desensitization may be due to brief inapproachability of the receptor to the agonist. Phosphorylation of GPCR by specific GRKs is the main mechanism for rapid desensitization. Phosphorylation of agonist-occupied GPCRs facilitates binding of cytosolic proteins like *arrestins* to the receptor and ends in uncoupling of the G protein[64].

**Image 5: Desensitization of GPCR[148]**




### 3) Infections

Viral infections like rhinovirus are general causes of asthma exacerbations. Children who suffer respiratory syncytial virus (RSV) infection may have a higher chance of developing asthma in later age. Atypical bacteria like *Chlamydophila* have also been thought to be related to asthma.[65]

**4) Diet**

Studies have shown that low antioxidants[66] or high sodium and omega-6 PUFA[67] are associated with an increased risk of asthma. Vitamin D deficiency[68] may also be associated with development of asthma. However, interventional studies with supplementary diets have not supported these facts.

**5) Air Pollution**

Air pollutants such as $SO_2$and ozone may trigger asthma symptoms. Indoor air pollution may be more problematic than outdoor pollution because of nitrogen oxides from stoves and passive smoking[69, 70, 71, 72].

**6) Occupational Asthma**

It affects nearly 10% of young adults[74]. Occupational triggers include exposure to animals in lab workers, fungal amylase in bakers. The diagnosis can be confirmed when symptoms improve on weekends[75].

**7) Obesity**

Asthma is highly associated with obesity (BMI>30 kg/m2) and it has been shown to be associated with poor disease control. Although mechanical factors may contribute to this, it could also be linked to the pro-inflammatory adipokines and reduced anti-inflammatory adipokines that are released from fat stores[76].

**8) Other Factors**

- Lower maternal age[77]
- Duration of breast-feeding[78]
- Prematurity &low birth weight baby[79]

- Inactivity.

**INTRINSIC ASTHMA**

This is a non-atopic form of asthma. These patients usually have adult-onset asthma, sometimes associated with aspirin sensitivity and nasal polyps. They usually have more severe, persistent asthma[80].

**ASTHMA TRIGGERS**

***1) Allergens***

One common allergen to increase symptoms is *Dermatophagoides* species &frequent exposure causes chronic symptoms. Animal allergens like hair from cats and other pets also worsen symptoms. Some allergens are seasonal like pollen, weed, and spores.

***2) Viral infections***

Rhinovirus, [81]Respiratory Syncytial Virus, [82]Coronavirus[83] is the most common triggers of acute severe exacerbations.

***3) Pharmacologic agents***

Beta-adrenergic blockers, ACE inhibitors, Aspirin sensitivity[84]

***4) Exercise***

Exercise induced hyperventilation can cause bronchial smooth muscle constriction. EIA typically starts at the end of exercise and symptoms reduce in a few minutes. It worsens during climate changes. [85]

***5) Physical factors***

Cold air[86] and hyperventilation[87] may trigger asthma. Laughter[88] is also another factor. Weather changes also trigger asthma.

***6) Food and diet***

Shellfish, nutscan sometimes causewheeze. Some additives can worsen the symptoms of asthma. Preservatives like Metabisulfite&tartarazine can cause triggering of symptoms[89].

**7) *Hormones***

There can be premenstrual worsening of symptoms, possibly due to reduction in progesterone.Thyrotoxicosis[90]or hypothyroidism[91]can also increase symptoms.

***8) Gastro Oesophageal reflux disease***

GERD is quite frequent in asthmatics, sometimes due to drugs[92]. Reflux of acid itself can cause bronchoconstriction[93]

**9) *Stress***

Asthma symptoms can worsen during periods of stress[94].Psychological factors can induce bronchial constriction through cholinergic pathways.

## CLINICAL FEATURES & DIAGNOSIS

The main symptoms of asthma are wheezing, dyspnoea, and cough, which are sometimes worse at night and during the early morning hours. Mucus secretion will be increased.[95]There can also be hyperventilation. Prodromal symptoms can also occur.

Typical signs include an inspiratory wheeze & hyperinflation. Children sometimes have cough-variant asthma[96].

**DIAGNOSIS**

**1) Pulmonary Function Tests**

- There will be reduced PEF,FEV1and FEV1/FVC ratio
- Diurnal variations can be confirmed by twice daily PEF
- Reduced peak flow and MEF in flow volume loop[97, 98]
- Whole-body plethysmography[99] - there will be a rise in resistance of airways, TLC and RV will be increased

**2) Hematologic Tests**

RAST[101] is done to measure the level of serum IgE.

**3) Imaging**

- Roentgenogram is mostly normal, but in patients with more severe disease, it may show hyperinflated lungs.
- High-resolution computed tomography (CT)– rarely bronchiectasis; thickening of airway walls in severe asthmatics. [102]

**4) Skin Tests**

May show positive results in allergic asthma due to inhalant allergens[103]

**5) Exhaled Nitric Oxide**

Fractional exhaled nitric oxide (FENO) is now being used as a non-invasive test to measure bronchial inflammation. The typically elevated levels in asthma are reduced by corticosteroids. [104]

## CURRENT TREATMENT

The goal of asthma therapy should be as follows[105]

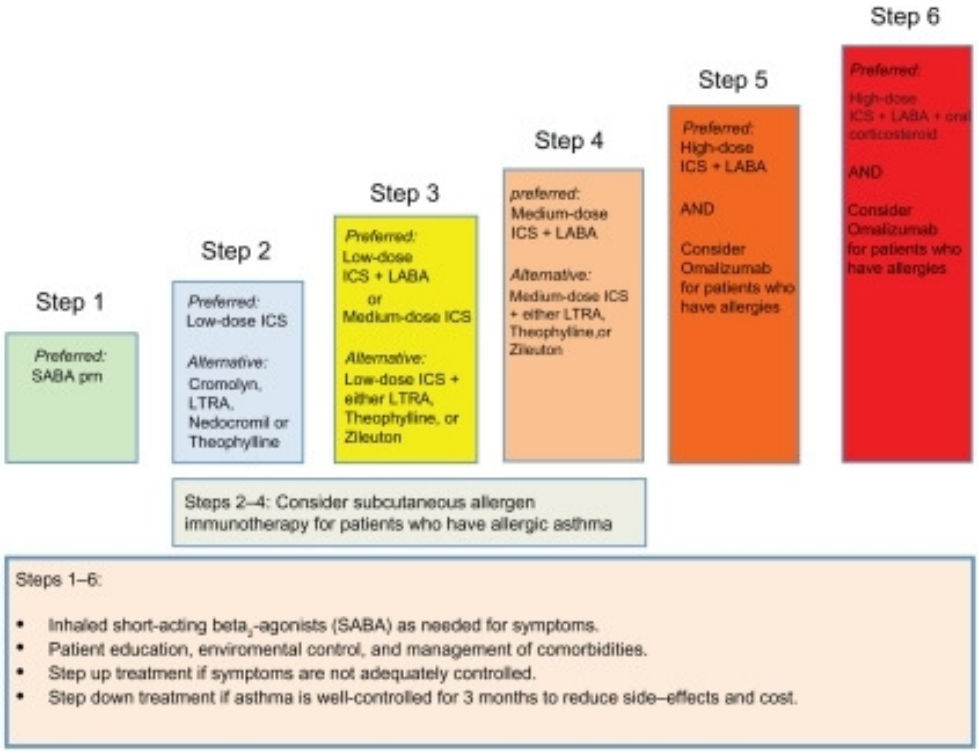
- **Minimal (ideally no) chronic symptoms including nocturnal**
- **Minimal (infrequent) exacerbations**
- **No emergency visits**
- **Minimal (ideally no) use of required $\beta_2$ agonist**
- **No limitations on activities, including exercise**
- **Peak expiratory flow circadian variation <20%**
- **(Near) normal pea k expiratory flow**
- **Minimal (or no) adverse effects from medicine**

Recommendations by the National Asthma Education & Prevention Program (NAEPP)-3 stresses on regular treatment with anti-inflammatory drugs like corticosteroids through inhaled route as the main point of management of severe bronchial asthma.

The management includes chronic controller medication and short term reliever medications. Long term medications include corticosteroids, long acting bronchodilators (LABAs), anti cholinergics, PDE inhibitors, leukotriene modifiers, desensitization with single allergen therapy, omalizumab and vaccination.

Short acting reliever medications include beta agonist, high dose corticosteroids and antimicrobials with respiratory support if necessary.

**Image 6: Stepwise treatment of Asthma**




## CONTROLLER THERAPIES

**S**teroids are the most active drugs currently in use. These drugs cut back acute & chronic inflammation, leading to reduction in the signs of respiratory disease, increase in flow of air, attenuation of airway hyper-responsiveness and decreased asthma recurrences. Such drugs additionally enhance the effect of beta agonists.

## CORTICOSTEROIDS

Oral corticosteroids were used in management of asthma since the 1950s and are the most effective controller therapy for asthma. The introduction of ICS helped in decreasing the use of oral steroids[106].

**Mechanism of Action**

Corticosteroids, after entering target cells, bind to glucocorticoid receptors (GR).The steroid-GR complex binds to certain genes in the nucleus, which causes transcription. They also react with transcription factors and co activators and so influence protein synthesis. The repression of transcription factors AP-1 and NF-κBmay be the basis of their anti-inflammatory properties. Additional mechanisms may include inhibitory action on mitogen activated protein (MAP) kinase pathways by inducing MAP kinase phosphatase (MKP-1) and inhibition of many genes.[107]

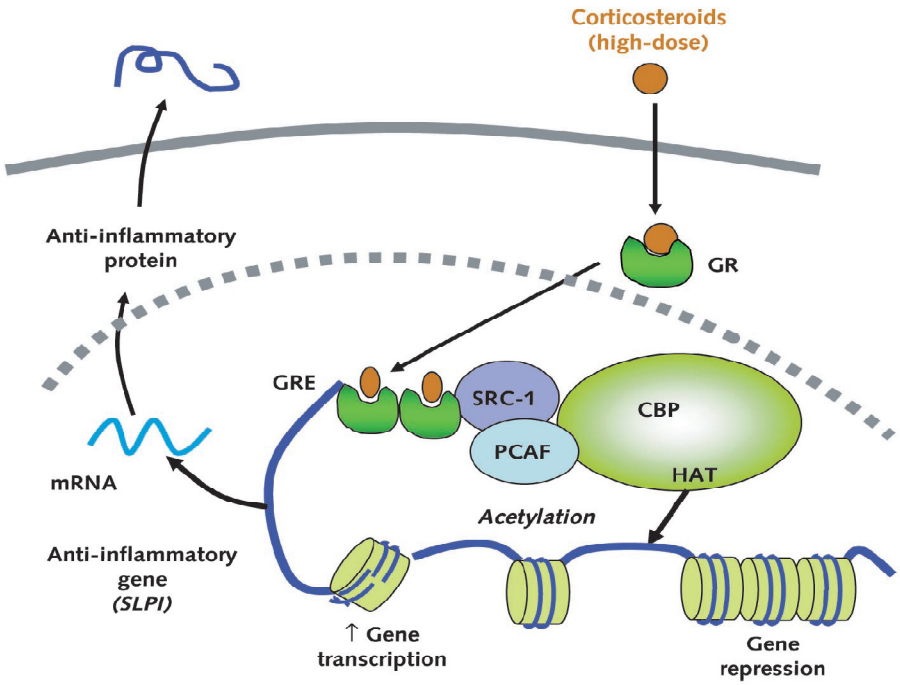
**Anti-Inflammatory Effects in Asthma**

Corticosteroids affect gene transcription. They stimulate anti-inflammatory and suppress pro-inflammatory genes. They have an additional inhibitory effect on active mediators. Damaged epithelium is also healed. Cytosolic GR bind with steroids; the receptor-ligand complexes translocate into the nucleus and inhibit histone acetyl transferase (HAT) activity in two ways: directly and, more importantly, by recruiting histone deacetylase (HDAC2). Steroids also inhibit cytokine production (e.g., IL-3, 4, 5, 9, 13, TNF-α & GM-CSF). They also decrease survival of eosinophils.

Corticosteroids have little action on the contractility of the airway smooth muscle (ASM). Hence decrease in symptoms may be due to action on chronic inflammation and AHR. ICS have quick effects, reducing AHR and concentration of mediators in sputum within a few hours[109]. It is to be

understood that corticosteroids *decrease* bronchial inflammation but do not cure it.

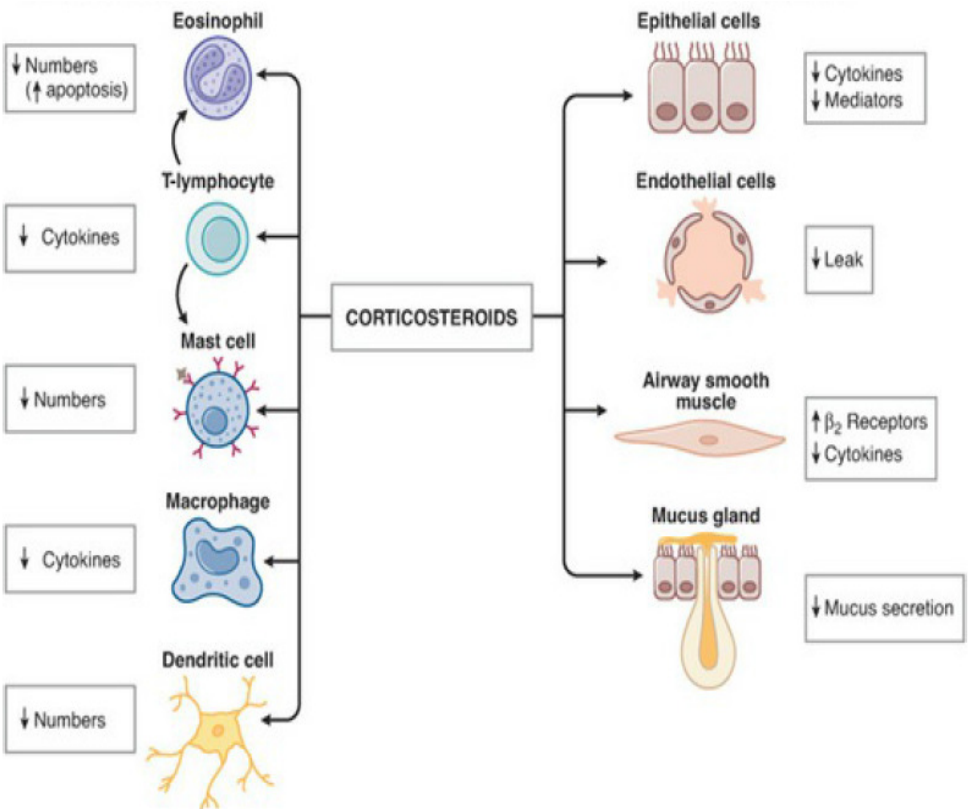
**Image 7: Mechanism of anti-Inflammatory Effect in Asthma**[108]




**Effect on $\beta_2$ Adrenergic Responsiveness**

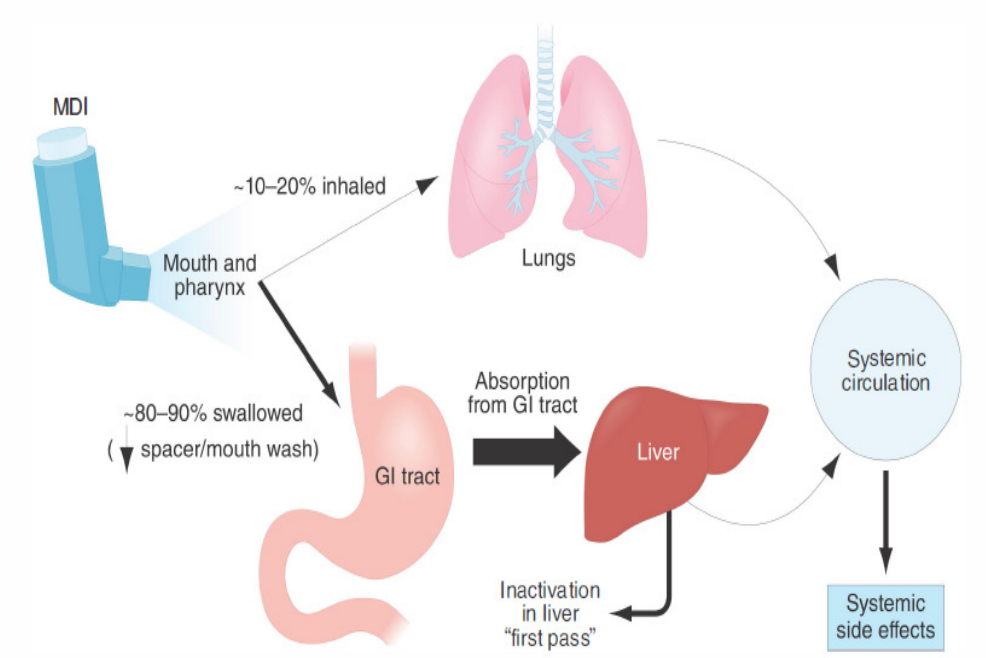
Corticosteroids increase the responsiveness of β adrenergic receptors and the increase the effects of beta agonists on ASM. They prevent and reverse β receptor desensitization[111,112].They also reduce uncoupling of $\beta_2$ receptors to Gs. In animals, they have been shown to reduce down-regulation of $\beta_2$ receptors. Likewise $\beta_2$ Agonists also enhance the action of GR, by increasing nuclear translocation of the ligand-GR receptor complex. This has been demonstrated in the sputum of asthmatics after ICS and inhaled LABA[113].

**Image 8: Effect of Corticosteroids on Airways**[110]




## Pharmacokinetics

Drug inhaled into the lungs acts locally on the mucosa. A small portion of drug may be absorbed systemically from the pharyngeal and bronchial surface. Use of spacer with MDI decreases the systemic absorption of ICS.Beclomethasone and ciclesonide are available as pro-drugs that release the active component locally. Budesonide and fluticasone have a higher first-pass effect than beclomethasone.




**Routes of Administration &Dosing of Inhaled Corticosteroids in Asthma**

ICS are the initial therapy for all patients with chronic asthma. They may be prescribed for an asthmatic who need twice weekly beta agonists for control. They are used in all types of asthma and in all age groups [114,115].

When ICS is used twice daily it improves patient compliance. Once daily administration is effective only when doses ≤400 µg/day are used. If a dose >800 µg is used, a spacer device should be used to reduce side effects. The least possible dose must be used to reduce symptoms[116]. Nebulizer steroids are used in small children who can't use inhalers.

**Systemic Steroids**

IV steroids are used in acute exacerbations with PFT <30% predicted and when asthmatics are not responding to beta agonist. Hydrocortisone is most commonly used due to its rapid onset of action. It can be switched to oral

prednisolone later. The usual maintenance dose is 10-15 mg/day. Short courses are advised for exacerbations; dose should be tapered before stopping.

**Side Effects:**

**Local:**

- Dysphonia
- Oropharyngeal candidiasis
- Cough

**Systemic:**

- Osteoporosis
- Cataracts
- Glaucoma
- Metabolic abnormalities (glucose, insulin, triglycerides)
- Psychiatric disturbances (euphoria, depression)
- Growth suppression
- Adrenal suppression and insufficiency

**Therapeutic Choices**

Inhaled steroids now available are

- Beclomethasone
- Budesonide
- Flunisolide
- Ciclesonide
- Triamcinolone
- Fluticasone

- MometasoneFuroate

All ICS are absorbed from the lung into the systemic circulation. Triamcinolone and flunisolideare the least potent, beclomethasone and budesonide are of equal potency; fluticasone is twice as potent as beclomethasone.

Several studies[150] have classified patients who receive inhaled corticosteroids for a period greater than 3 months as requiring "long term therapy"

The potency and dosage of individual drugs of inhaled corticosteroids are well defined in the GINA guidelines for asthma 2015 and are given in the table below .[149]

**Image 10: Inhaled corticosteroids Dose equivalents**

| Drug | Low dose | Medium dose | High dose |
|---|---|---|---|
| Beclomethasone dipropionate | 200 ~ 500 | >500 ~ 1,000 | >1,000 ~ 2,000 |
| Budesonide | 200 ~ 400 | >400 ~ 800 | >800 ~ 1,600 |
| Ciclesonide | 80 ~ 160 | >160 ~ 320 | >320 ~ 1,280 |
| Flunisolide | 500 ~ 1,000 | >1,000 ~ 2,000 | >2000 |
| Fluticasone | 100 ~ 250 | >250 ~ 500 | >500 ~ 1,000 |
| Mometasone furoate | 200 ~ 400 | >400 ~ 800 | >800 ~ 1,200 |
| Triamcinolone acetonide | 400 ~ 1,000 | >1,000 ~ 2,000 | >2,000 |

**Corticosteroid-Resistant Asthma**

This happens due to molecular abnormalities causing impaired anti-inf1ammatory action of corticosteroids. Complete resistance is very rare. Several mechanisms that have been described like

- ✓ Rise in the spliced form of glucocorticoid receptor
- ✓ Atypical histone acetylation
- ✓ Defective IL- 10 production
- ✓ Reduced HDAC2 activity

**BRONCHODILATOR DRUGS:**

Their focus is mainly on ASM to reduce bronchial constriction giving a quick relief from symptoms[117]. Three classes are there namely

- ✓ ß2-adrenergic agonist – more effective[118]
- ✓ Anticholinergics[119]
- ✓ Theophylline[120]

**ß2-Agonists[121]**

They activate ß2-adrenergic receptors present in airways; they couple with $G_{s}$-protein to adenylyl cyclase there by increasing cAMP, which causes smooth muscle relaxation and inhibition of mast cells. Commonly used long acting ß2agonists include salmeterol and formoterol. They are available as dry powder& are administered using delivery devices. These long acting drugs are not used as monotherapy, as they lack anti-inflammatory property. The effectiveness of LABA and ICS has led to using them in combination medication that delivers both agents at the same time. For acute exacerbation, short acting drug are the most effective, which includes albuterol, levoalbuterol, terbutaline, pirbuterol etc.

These drugs relax the airways by relaxing the smooth muscle and by increasing the airflow, thereby relieving the bronchoconstriction and reducing the symptoms. Inhaled β-adrenergic drugs are as effective as oral or parenteral therapy. Severe exacerbations often need higher doses.

**Anticholinergics**[121]

Anticholinergics such as ipratropium bromide and tiotropium bromide reduce bronchial constriction and mucus secretion. When compared to beta agonists, these are less effective, the reason being specific action on bronchoconstriction mediated through cholinergic activation unlike β-adrenergic drugs which are functional antagonists to various bronchoconstrictor stimuli.

**Theophylline**[121]

Theophylline is an oral bronchodilator, now not used frequently due to side effects and other drugs with better efficacy. Mechanism of action is mainly through PDE inhibition in ASM. It inhibits HDAC2 by switching off active inflammatory genes. They are given as once or twice daily orally. Aminophylline is rarely used. The most common side effects are nausea, vomiting and headache.

## OTHER CONTROLLER THERAPIES

### Leukotriene inhibitors:

Inhibiting **t**he LT pathways may be valuable in the management of asthma, which also led to the development of 5′-LOX inhibitors – zileuton, cys-LT1 receptor, inhibitors like montelukast, zafirlukast. They are used as add-on therapy in patients who are not well controlled on ICS[122]. They do not appear to provide any additional benefit in steroid resistant patients [123].

### Anti-lgE antibody

Omalizumab neutralizes circulating IgE and inhibits IgE-mediated reactions[124]. It decreases exacerbations and is used in patient not controlled by other modes of treatment. Omalizumab is given as a subcutaneous injection every 2-4 wks[125, 126.]

## FUTURE THERAPIES

### Anti-interleukin 4 antibody[127-130]

- IL-4 binds to receptor α subunit

  -type I receptors bind only to IL 4

  -type II receptors binds to both IL 4 &13

- Treatment with Dupilumab(Phase II), a human monoclonal antibody binds to interleukin-4 receptor α subunit , reduces exacerbation rates
- Pitrakinra(Phase II) an interleukin-4 variant
- Pascolizumab development was stopped early because there was no evidence of clinical benefit

**Anti-interleukin-5 antibody**[131-134]

- Mepolizumab (Phase III)

- Reslizumab (Phase II)

-↓ exacerbations and ↓ use of oral corticosteroids,

-without improvement in FEV1

**Anti-interleukin-13 antibody**[135, 136]

- Interleukin 13 like IL15 also induces corticosteroid insensitivity
- Lebrikizumab (Phase II) an antibody to IL13,

  -improved FEV1 in moderate to severe asthma

  -did not affect exacerbations or asthma symptoms

**Anti-CD25 antibody**[138]

Daclizumab, a humanised immunoglobulin G1 monoclonal antibody

- ✓ improved FEV1 and asthma control in adults

**Tyrosine kinase inhibitor**[137]

- Masitinib, inhibitor of tyrosine kinase that inhibits stem cell factor receptor (c-kit) and PDGF

  - ↓ the dose of oral corticosteroids required

  - No effect on lung function

**ANTIMICROBIALS**[139, 140]

Various studies have demonstrated that infection with viruses especially rhinovirus and bacteriae like mycoplasma or chlamydia may lead to acute exacerbation. Hence usage of antibiotics has been suggested during these exacerbations. Regular usage of antibiotics in the absence of exacerbation is

not recommended, as there are no consistent testimonies which claim to improve clinical outcome with antibiotic usage. Antibiotics ought to be started once there is a high probability of evidence for respiratory tract infections like pneumonia, fever and sinusitis.

**TOOLS USED:** ACT: Asthma control test questionnaire:

There are multiple methods used to know the range of control in asthmatics. ACT and ACQ are the commonly used tests. They're symptom based evaluation type of questionnaire while others are based on pulmonary function test. ACT in specific has been found to be helpful for primary health care development in developing countries[94].

Accurate assessment of control is a very important a part of asthma management. Inadequate assessment of control could be a major issue for poor control of asthma[95].Asthmacontrol test (ACT) is a self-administered tool for assessing patient's control. It comprises 5 item questionnaires which deals with symptoms and difficulty in daily activities are recalled for 4 wks, need of control medication, and self-rating of control[101].

It has been made known to show a relationship with a physician's ratings of control[102]. An ACT score >19 indicates well-controlled asthma. ACT has conjointly been validated to be used by mail[103] and by telephone[104].

The ACT is straightforward to use and provides a numeric score which will assist in assessing control over time[102]. Better assessment of control could lead to improved status of patient, awareness, understanding control, and to improve patient outcomes.

## JUSTIFICATION FOR THE STUDY

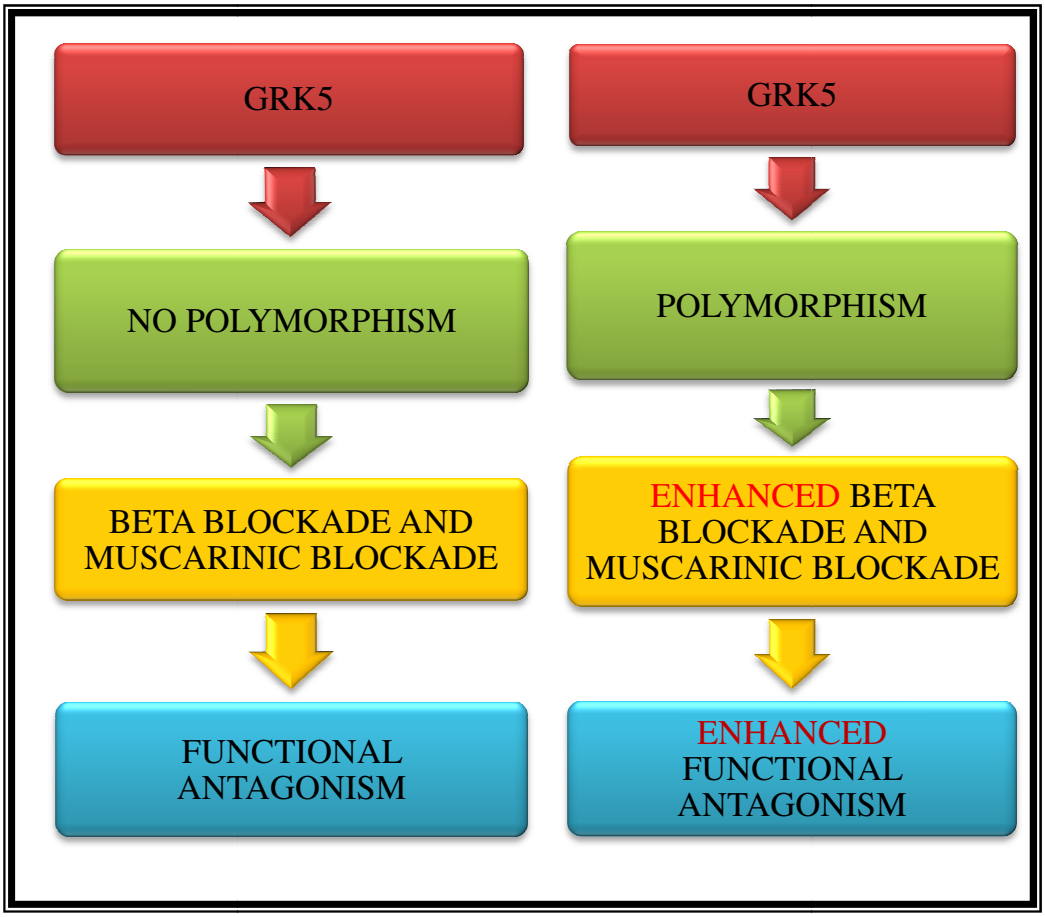
From the above discussion about GRK5 it is clear that this has an important role in asthma. GRK5 enzyme is controlled by a gene – the GRK5 gene. An SNP in this gene, at position 41,results in the amino acid glutamine (Gln) being changed to leucine(Leu).This polymorphism causes augmented receptor desensitization and internalization, which causes various effects in various organs[141]. In relation to asthma, this polymorphism causes beta adrenoceptor desensitization leading to enhanced beta blockade, which in turn decreases the control of asthma[142].

Some studies show that muscarinic receptor super sensitivity can also occur due to this GRK5 polymorphism this can also contribute to poor control in asthmatics. GRK5 therefore, regulates pulmonary response in a tissue specific and receptor specific manner[143].

Inhaled steroids are highly potent inhibitors of the process of inflammation and are commonly used in the management of asthma. They also inhibit mediators like cytokines, chemokines and adhesion molecules[144] .A study was done in rats to determine how glucocorticoids regulated GRK5 expression. This study revealed that the activity of cytosolic GRK and the expression of GRK5 was elevated in the lung of IL-1β-treated rats, and that this was completely abolished by dexamethasone. Though dexamethasone only partially reversed the loss of IL-1β-induced relaxation activity, it caused

complete inhibition of IL-1β-induced increase in GRK activity and protein expression[145].

From the above studies it is clear that glucocorticoids have an effect on the regulation of GRK5 by reversing the action of IL-1β and hence there is a possibility of variation in response to glucocorticoids in asthmatics with GRK5 polymorphism. There are very few studies exploring GRK5 in relation with asthma. No studies have been done so far exploring the effect of the polymorphism on the response to inhaled glucocorticoids.



# *Methodology*

# METHODOLOGY

## STUDY CENTRE:

This study was done at Department of Pharmacology, PSG Institute of Medical Sciences and Research and the Department of Pulmonology and Respiratory Medicine, PSG Hospitals, in collaboration with the PSG Centre for Molecular Medicine and Therapeutics (CMMT).

## STUDY SUBJECTS:

Inpatients and outpatients of Respiratory Medicine and Pulmonology department with clinical diagnosis of asthma from June 2014 to July 2015

**STUDY LOCALE:** PSG Hospitals -Coimbatore

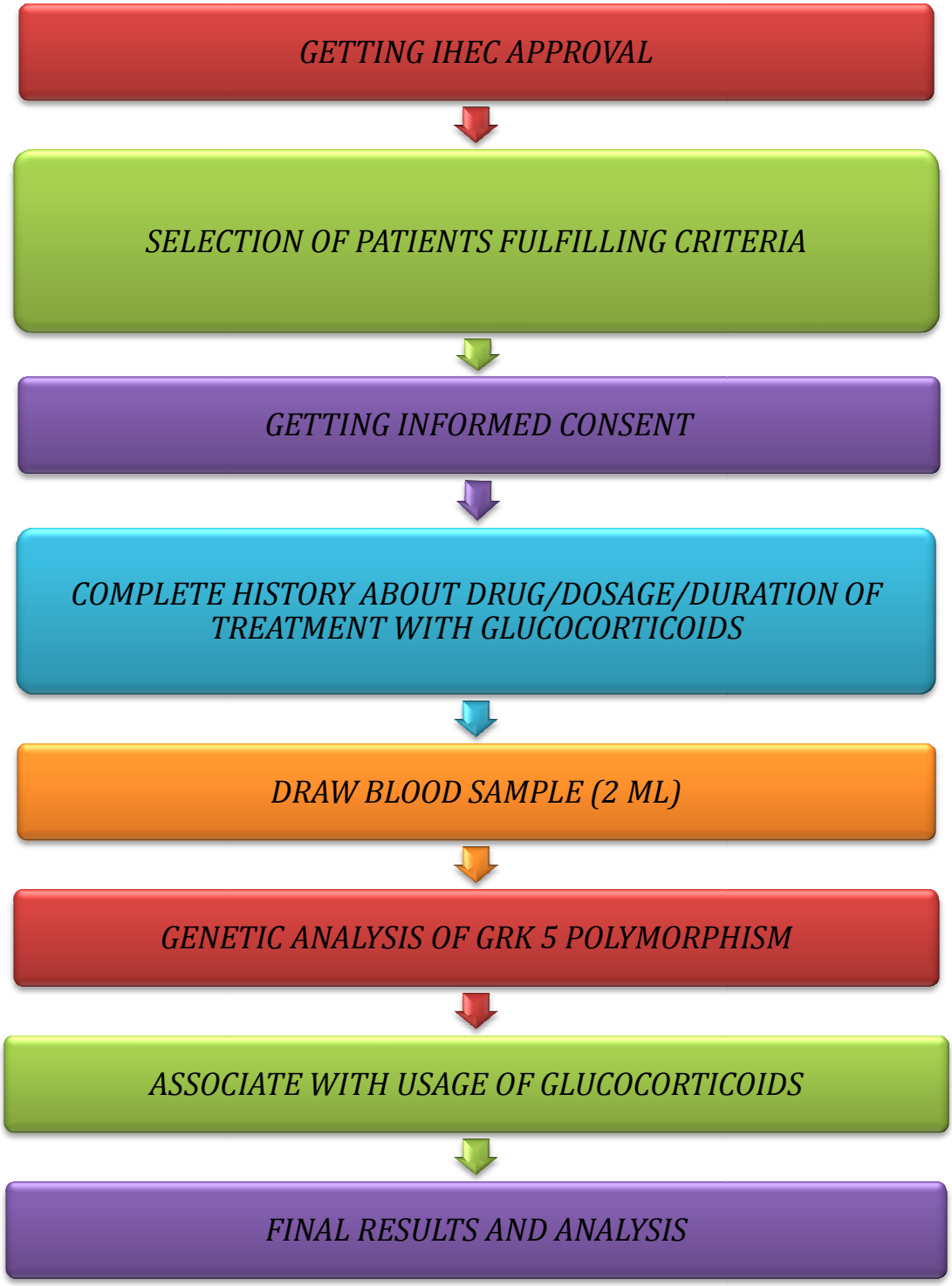
## STUDY DESIGN:

The study was a cross sectional study. After obtaining written informed consent from the patients fulfilling the inclusion & exclusion criteria, the study participants were given the self-administered questionnaire in the colloquial language in order to ensure that they understood the questions before giving appropriate responses. During the same visit, blood samples were collected from all the patients and genetic analysis was done.

## SAMPLE SIZE:

Sample size of 149 patients who were on inhaled steroids for treatment of asthma was chosen for the study.

**Picture: FLOWCHART OF STUDY DESIGN**



## ETHICAL APPROVAL:

The study protocol was accepted by the Institutional Human Ethics Committee (IHEC) preceding the start of the study. The details and the purpose of the study protocol were explained to each participant individually and their doubts were clarified before obtaining informed consent. The informed consent forms were provided to the participants either in English or Tamil depending on the participant. The participants, who gave written informed consent, and eligible for recruitment according to the inclusion criteria, were enrolled for the study. A copy of the consent form is attached in the annexure.

## TOOL USED

- Case report form
- **Asthma control test** - a validated self-administered questionnaire was used to assess control of asthma
    - **Score more than 19 – Well controlled**
    - **Score less than or equal to 19 – Poorly controlled**

Patients were classified as those requiring high dose or low dose inhaled steroid based on GINA guideline for Inhaled steroids Dose equivalent table.

The duration of treatment was also classified into long term or short term. If the patient was on inhaled steroids for more than 3 months, they were categorized as long term users of inhaled steroids while those with less than 3 months of use were grouped as those on short term treatment.

## INCLUSION CRITERIA

- All patients diagnosed to have asthma according to the accepted guidelines and were under treatment with corticosteroids.
- Ability and willingness to provide written informed consent
- Age >12 years

## EXCLUSION CRITERIA

- Asthma patient not on inhaled corticosteroids

## GENETIC ANALYSIS:

Steps involved in genotype analysis were;

- DNA extraction
- TETRA - PCR to amplify GRK 5 gene
- 2% Agarose gel electrophoresis

### Blood Collection and Treatment

For every 1 ml of whole blood sample, add 0.1 ml of anticoagulant (0.5M EDTA pH 8.0).

### Storage of Blood

Whole blood samples treated with EDTA can be used, either fresh or frozen. For short term storage (up to 10 days), collect blood in tubes

containing EDTA as an anticoagulant, and store tubes at 2-8ºC. It is recommended to store blood samples less than 3 days as DNA degradation may occur. For long term storage, collect blood in tubes containing a standard anticoagulant (preferably EDTA if high molecular weight DNA is required) and store at -80ºC.

## DNA EXTRACTION

**Procedure for Extraction Genomic DNA from Blood was done using Kit (EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood) and the steps followed were the following:**

**1. Sample Preparation:**

a. Blood Samples (non-nucleated Erythrocytes, for example Human Blood): Collect ~100ul of blood into 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200µL. Vortex softly and let tube stand for 1 minute at room temperature.

   i. If >100ul of blood is used, add 2 volumes of Buffer TBP. Mix thoroughly and make tube stand for 1 minute until red cells lyse completely. Spin at 4,000 *x* g (8,000 rpm) for 1 min. Discard the supernatant carefully. Wash the precipitate with 500µl TE Buffer 2 times. Spin at 4,000 *x* g (8,000 rpm) for 1 min during each wash. The final precipitate should appear white. Proceed with step 2.

   ii. Typical Yield is 1-3µg from 100ul blood sample.

b. Blood Samples (Nucleus-containing Erythrocytes, for example chicken Blood): Collect ~10µl of blood into a 2.0 ml centrifuge tube. Add PBS solution to the tube to a final volume of 200ul. Vortex lightly and allow tube to stand for 1 minute at room temperature. Proceed to step 2.

c. Solidified Blood Clot: Weigh 0.1g of blood. Grind to find powder under liquid nitrogen. Add 200ul of PBS solution and proceed to step 2.

2. Add 20µl of proteinase K. Mix well. Add 200 µl of Buffer CL. Vortex Gently. Incubate at 56ºC for 10min.

- ✓ The solution should appear clear after complete lysis. If solution still appears cloudy, extend incubation time until lysis is complete and solution is clear.
- ✓ If RNA-free genomic DNA is necessary, add 20 µl RNase A (10 mg/ml, not provided with kit), and mix by vortexing, and incubate for 5 min at room temperature before doing with step 3.
- ✓ If final reaction volume is more than 500ul, increase proteinase K usage and/or extend incubation time.

3. Add 200ul of 100% ethanol to the mixture and mix thoroughly.

- ✓ Small cloudy insoluble material may appear after addition of ethanol, but this does not affect the performance of the kit. Proceed with step 4.

4. Transfer the mixture from step 3 (including any precipitates) into an EZ-10 column that is in a 2.0 ml Collection Tube. Let it stand at Room Temperature for 1-2min. Spin at 8,000 *x g* (10,000 *rpm*) for two minutes. Throw away the flow-through in the collection tube.

5. Include 500 µl of CW1 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for one minute. Next, ensure ethanol has been added to the CW1 concentrate prior to usage.

6.Add 500 µl of CW2 Solution, and spin at 8,000 *x g* (10,000 *rpm*) for 1 minute. Next, ensure ethanol has been added to the CW2 concentrate prior to usage.

7. Throw away the flow-through. Spin at 8,000 *x g* (10,000 *rpm*) for an additional minute to remove any residual amount of CW2 Solution.

8.Place the column into a clean 1.5 ml Eppendorf tube. Add 30-50 µl CE Buffer into the center of membrane within the column. Incubate at RT for 2 to 3 minutes. Incubating the tube at 37ºC or 50ºC for 2 minutes may increase recovery yield.

9. Spin at 8,000 *x g* (10,000 *rpm*) for 1 minute to elute DNA from the column.

10. For long term storage, keep aliquots of purified genomic DNA at -20□C.

11. Measure DNA quantity by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg).

## GRK 5 TETRA PRIMER PCR STANDARDISED PROTOCOL

### REQUIREMENTS:

### 1. PRIMERS (SIGMA)

| S.NO | PRIMERS | 2° STOCK CON | FINAL CONCENTRATION |
|---|---|---|---|
| 1 | FORWARD PRIMER 1 GRK5F | 1µM | 35 nM |
| 2 | REVERSE PRIMER 1 GRK5R | 1µM | 35 nM |
| 3 | FORWARD PRIMER 2 GRK5SNPTR | 1µM | 45 nM |
| 4 | REVERESE PRIMER 2 GRK5SNPTF | 1µM | 45 nM |

### 2. DNTPs (HIMEDIA)

| S.NO | DNTPS STOCK | 1°STOCK CON | 2° STOCK CON | FINAL CON |
|---|---|---|---|---|
| 1 | 100 mM each DNTPS | 10 mM each DNTPS | 2.5 Mm each DNTPS | 100µM each DNTPS |

3. **Taq DNA polymerase** (colour Taq Genei) (Stock 1 Unit/ µl, Final con 0.1 unit)
4. **Taq Buffer A** (Genei) (Stock 15mM $Mgcl_2$, Final con 1.5mM$Mgcl_2$)
5. **DNA Sample** Optimized concentration: 0.03µg/ 20µL
6. **Milliq water**

**PCR REACTION MIX:**

| S.NO | COMPONENTS | VOLUME | FINAL CONC |
|---|---|---|---|
| 1 | Forward primer 1 - 1µM | 0.7µL | 35 nM |
| 2 | Reverse primer 1 - 1µM | 0.7µL | 35 nM |
| 3 | Forward primer 2 - 1µM | 0.9µL | 45 nM |
| 4 | Reverse primer 2 - 1µM | 0.9µL | 45 nM |
| 5 | Taq Buffer A | 2µL | 1.5 mM Mgcl2 |
| 6 | DNTPs | 0.8µL | 100µM each DNTPs |
| 7 | Taq enzyme | 0.5µL | 0.01 unit |
| 8 | DNA Sample(0.3µg) | | 0.3µg/20µL |
| 9 | Milli Q(H2O) | UPTO 20µL | |

**PCR PROGRAMME:**

1. **Initial denaturation - 94°C for 5 min**
2. **Denaturation 1 - 94°C for 30 sec**
3. **Annealing 1 - 65°C for 30 sec** } **15 CYCLES**
4. **Extension 1 - 72°C for 1 min**
5. **Denaturation 2 - 94°C for 30 sec**
6. **Annealing 2 - 55°C for 30 sec** } **27 CYCLES**
7. **Extension 2 - 72°C for 1 min**
8. **Final extension - 94°C for 5 min**
9. **Then held at 4°C**

- ***PCR amplification confirmed on 2% agarose gel***

## AGAROSE GEL ELECTROPHORESIS

Reagents required:

**1.** TAE buffer 50 X stock:

- 24.2gmTris base
- 5.7ml Glacial Acetic acid
- 10ml 0.5M EDTA pH 8.0
- Dissolve in 100 ml Distilled water

**2.** TAE buffer 1X Tank buffer:

Dilute the 50X TAE buffer to 1X by adding water. For eg (10ml 50X TAE + 490ml Water) it becomes 1X TAE. Use it for electrophoresis tank to run the gel.

**3.** DNA loading dye

**4.** Ethidium bromide.

**5.** Agarose powder.

**Gel preparation:**

- Take fresh 50ml 1X TAE buffer (based on gel template size) in a conical flask and add 1g agarose (2% gel), heat the solution well.
- Before that seal the Gel template with cello tape and keep the well template ready.
- Then, add 0.2µl ETBR solution with heated 50ml solution mix well and pour it in sealed gel template and leave it for a while to become a gel.

**Electrophoresis:**

- Load the 1X TAE buffer in tank (~600ml required) and keep the gel plate inside the tank.
- Then take 2µl DNA loading dye and 8µl DNA (isolated) mix well and load it in wells carefully.
- Set the volt at 65V and run it for 30-45 min. Then analyze the DNA bands using gel document machine.
- Quantification of the extracted genomic DNA was done using Nanodrop quantification after 2% agarose gel electrophoresis.

## GRK 5 RFLP PCR

**REQUIREMENTS:**

**1. PRIMERS (SIGMA)**

| S.NO | PRIMERS | 2° STOCK CON | FINAL CONCENTRATION |
|---|---|---|---|
| 1 | FORWARD PRIMER 1 GRK5BsrF | 1µM | 50nM |
| 2 | REVERSE PRIMER 1 GRK5BsrR | 1µM | 50nM |

**2.DNTPs(HIMEDIA)**

| S.NO | DNTPSSTOCK | 1°STOCKCON | 2° STOCKCON | FINAL CON |
|---|---|---|---|---|
| 1 | 100 mM each DNTPS | 10 mM each DNTPS | 2.5 Mm each DNTPS | 100µM each DNTPS |

3. **Taq DNA polymerase** (colour Taq Genei) (Stock 1 Unit/ µl, Final con 0.1 unit)

4. **Taq Buffer A** (Genei) (Stock 15mM $Mgcl_2$, Final con 1.5mM$Mgcl_2$)

5. **DNA Sample** Optimised concentration: 0.03µg/ 20µL

6. **Milliq water**

## RESTRICTION DIGESTION

**Requirements:**

1. BsrI Enzyme
2. 10 x Buffer R
3. PCR product

**Optimized Concentration:**

- **For wild Homozygous - 2GG**
- **For Mutant Homozygous - 2 GG**
- **For Heterozygous - 3 GG**

**REACTION MIX FOR RFLP:**

| S.NO | COMPONENTS | VOLUME |
|---|---|---|
| 1 | BsrI Enzyme | 1µL |
| 2 | 10X Buffer | 5µL |
| 3 | DNA Concentration | 1µG |
| 4 | Total Reaction Volume | 50µL |

- Mix gently & spin down for few seconds.

- Incubate at $65^0$C for 15-40 min.
- Restriction digestion product is inactivated at $80^0$C for 20 min before gel electrophoresis.

## POLYACRYLAMIDE GEL ELECTROPHORESIS

The DNA Fragments were separated using 15 % Polyacrylamide gel electrophoresis.

**Polyacrylamide gel prepared from the following reaction mix**

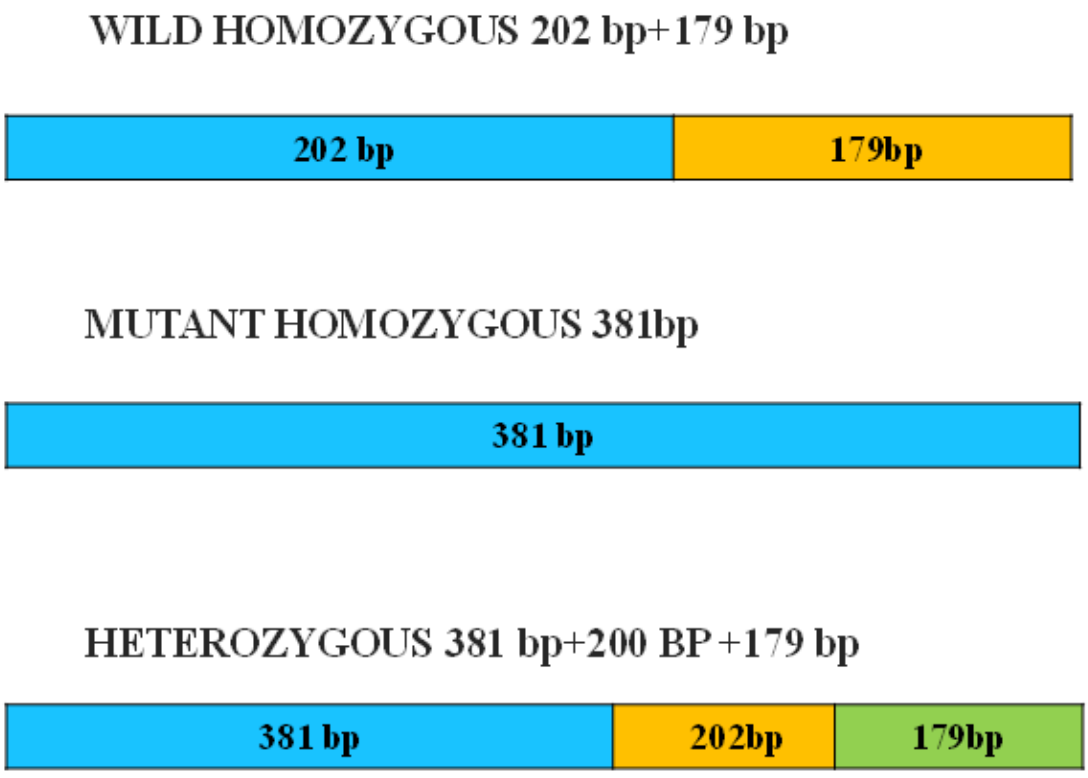
| 15% GEL | 5 ml | 10 mL | 15 mL | 20 mL | 25 mL | 30mL | 40mL | 50mL |
|---|---|---|---|---|---|---|---|---|
| WATER | 1.2 | 2.3 | 3.5 | 4.6 | 5.7 | 6.9 | 9.2 | 11.4 |
| A:B(30:8) | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 20 | 25 |
| 1.5M Tris pH 8.8 | 1.3 | 2.5 | 3.8 | 5 | 6.3 | 7.5 | 10 | 12.5 |
| 10% SDS | 0.05 | 0.1 | 0.15 | 0.2 | 0.25 | 0.3 | 0.4 | 0.5 |
| 10% APS | 0.05 | 0.1 | 0.15 | 0.2 | 0.25 | 0.3 | 0.4 | 0.5 |
| TEMED | 0.002 | 0.004 | 0.006 | 0.008 | 0.01 | 0.012 | 0.016 | 0.020 |

- Polyacrylamide gel was run in Amersham electrophoresis system at 100V for about 5 hours
- The gel was then stained with ethydium bromide
- The stained gel was viewed in a Chemiluminescence gel documentation system to identify the DNA fragments

**IDENTIFICATION OF GENOTYPES**

- Restriction was seen when '**A**' was present ie (Homozygous) Wild type thus produced 202 bp + 179 bp
- Restriction did not occur when '**T**' was present ie (Homozygous) Mutant type thus produced only 381 bp
- Restriction occurred when '**A**' & '**T**' both were present ie (Heterozygous)Wild type thus produced 381 bp + 202 bp + 179 bp

**Picture: Showing the Base pair size of the GRK 5 gene**



# *Results*

# RESULTS

## STATISTICAL ANALYSIS

Done using IBM-SPSS software version 22.The analysis done was **Chi Square Test** for analyzing the various factors influencing the requirement of high dose, duration of drug therapy and control of disease

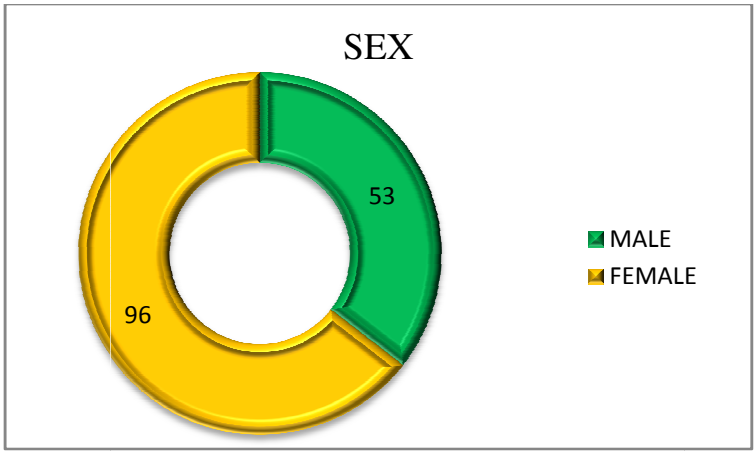
**Multivariate Regression analysis model** was used to analyze the influence of covariates on above mentioned factors in relation to inhaled steroid therapy in asthmatic patients.

## Distribution of Sex

**Table1: Distribution of Male and Female among asthmatics**

| SEX | | PERCENTAGE |
|---|---|---|
| MALE | 53 | 35.5% |
| FEMALE | 96 | 64.5% |
| TOTAL | 149 | 100% |

**Figure 1: Distribution of Male and Female among asthmatics**



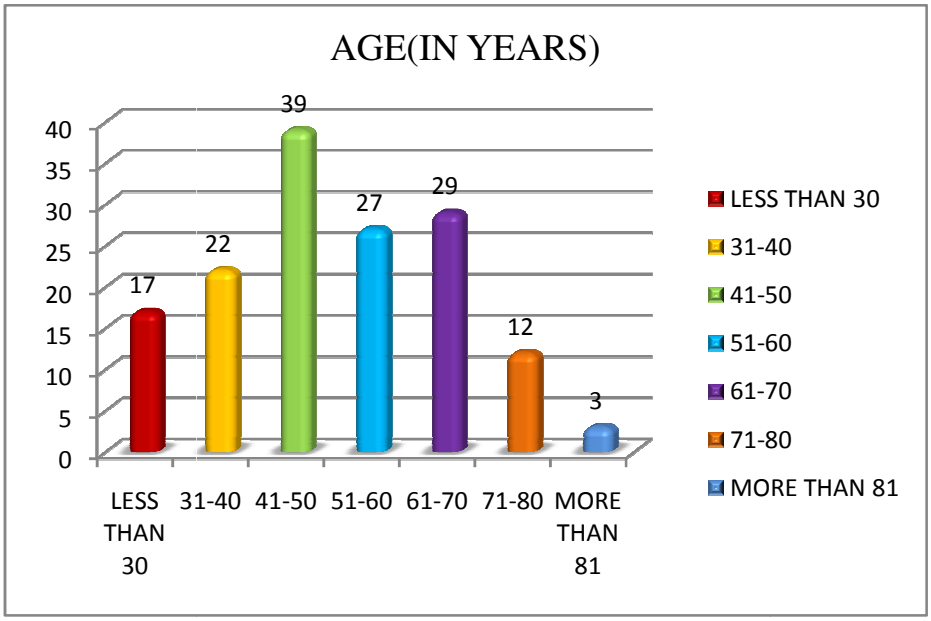

Among 149 patients 53(35.5%) were male and 96(64.5%) were female which showed a female preponderance at a ratio of 1:1.81.

## Distribution of Age

**Table 2: Distribution of Age among asthmatics**

| AGE(IN YEARS) | | PERCENTAGE |
|---|---|---|
| LESS THAN 30 | **17** | **11.5%** |
| 31-40 | **22** | **14.7%** |
| 41-50 | **39** | **26.2%** |
| 51-60 | **27** | **18.1%** |
| 61-70 | **29** | **19.5%** |
| 71-80 | **12** | **8%** |
| MORE THAN 81 | **3** | **2%** |
| TOTAL | **149** | **100%** |

**Figure 2: Distribution of Age among asthmatics**



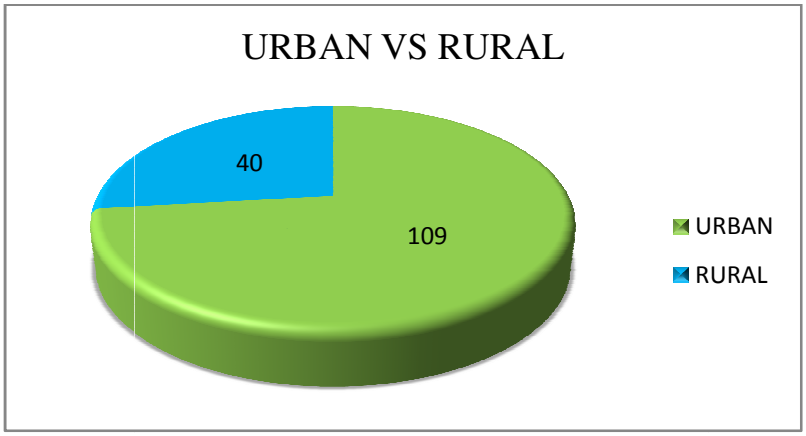

In our study, age of patients ranged from 18-87 years with mean of 50.5 ± 16.04 years. Patients were more commonly in the age group of 41-50 years - 39(26%) patients. In our study group, patients with age above 40 years (51%) were almost equal to patients below 40 years (49%). In addition, we found that there was no statistical significance (P=0.954) on influence of age over prevalence of asthma.

**Table 3: Incidence of asthma between Urban & Rural Population**

| URBAN Vs RURAL | | PERCENTAGE |
|---|---|---|
| URBAN | **109** | **73.2%** |
| RURAL | **40** | **26.8%** |
| TOTAL | **149** | **100%** |

Among our study group urban population was found to be more than rural patients which may be due to higher exposure to multiple reasons like air pollution, urbanization, etc. which are risk factors for asthma being more common in the urban population

**Figure 3: Incidence of asthma between Urban & Rural Population**



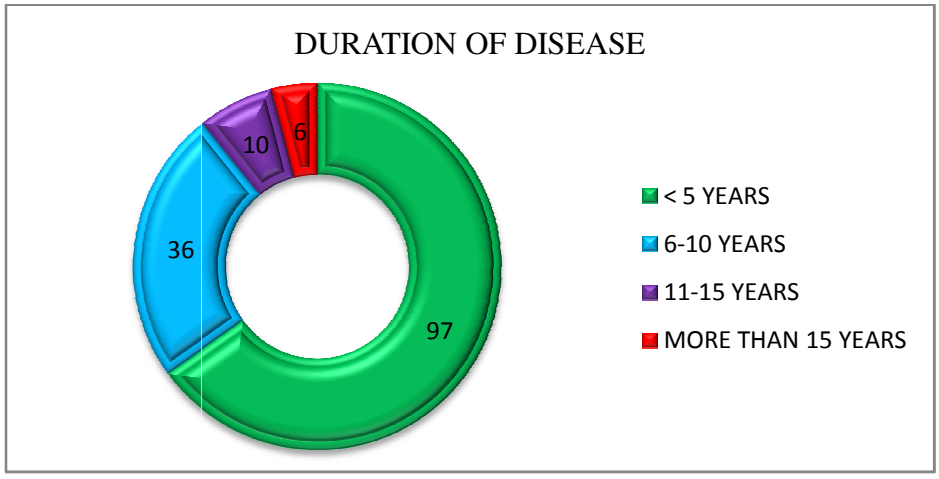

## DURATION OF DISEASE

**Table 4: Frequency of duration of disease**

| DURATION OF DISEASE | | PERCENTAGE |
|---|---|---|
| < 5 YEARS | **97** | **65.4%** |
| 6-10 YEARS | **36** | **24%** |
| 11-15 YEARS | **10** | **6.6%** |
| MORE THAN 15 YEARS | **6** | **4%** |
| TOTAL | **149** | **100%** |

In our study group most of the patients had asthmatic symptoms less than 5 yrs, whereas only few patients had disease more than 15 years.

**Figure 4: Frequency of duration of disease**




## TYPE OF INHALED STEROID

**Table 5: Type of steroid therapy**

| INHALED STEROIDS | | PERCENTAGE |
|---|---|---|
| BUDESONIDE | **53** | **35.5%** |
| BUDESONIDE/FORMOTEROL | **64** | **42.9%** |
| FLUTICASONE | **24** | **16.1%** |
| FLUTICASONE/SALMETEROL | **8** | **5.5%** |
| TOTAL | **149** | **100%** |

**Figure 5: Type of steroid therapy**

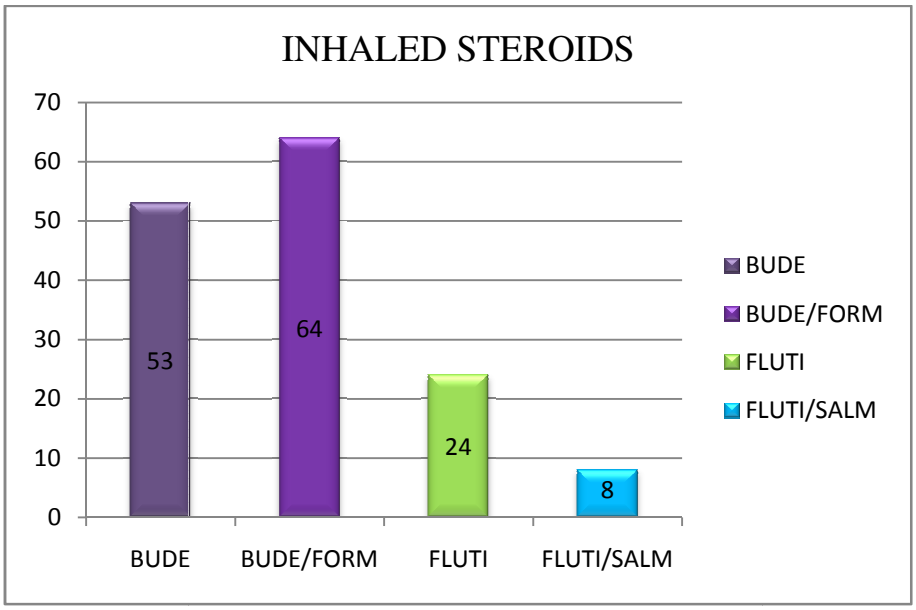
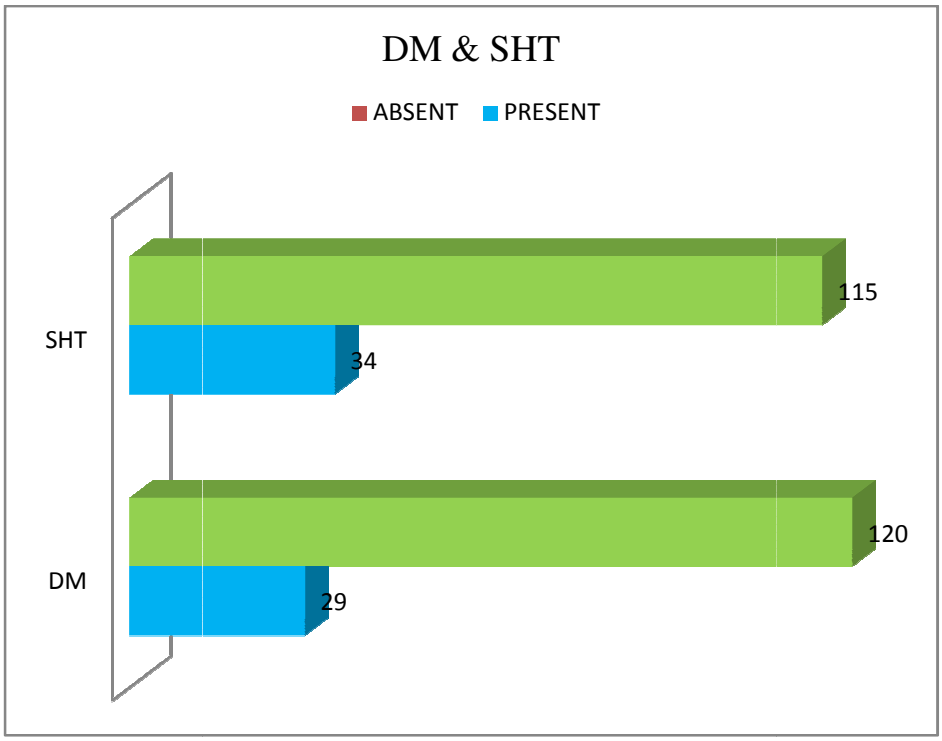


**Table 6: Prevalence of DM & SHT**

| CO MORBIDITIES | DIABETES MELLITUS | SYSTEMIC HYPERTENSION |
|---|---|---|
| PRESENT | **29(19.5%)** | **34(22.8%)** |
| ABSENT | **120(80.5%)** | **115(77.2%)** |
| TOTAL | **149(100%)** | **149(100%)** |

**Figure 6: Prevalence of DM & SHT**




In our study group 29 patients had diabetes mellitus and 34 patients had Systemic hypertension.

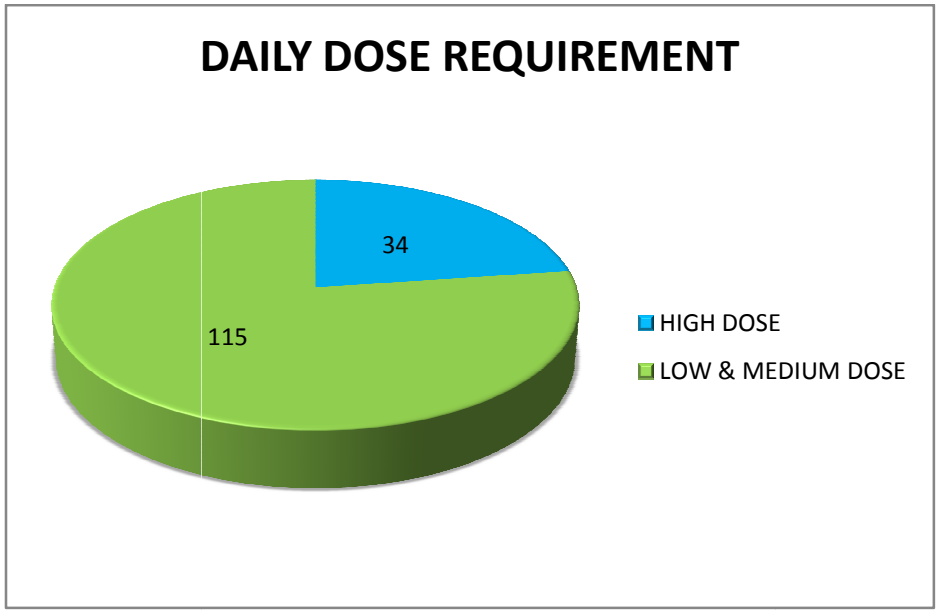
## DOSE REQUIREMENT

**Table 7: Requirement of high dose**

| DAILY DOSE REQUIREMENT | | PERCENTAGE |
|---|---|---|
| HIGH DOSE | **34** | **23%** |
| LOW & MEDIUM DOSE | **115** | **77%** |
| TOTAL | **149** | **100%** |

We analyzed patients requiring high dose inhaled steroids based on dosage guidelines in GINA report 2015 for different inhalational corticosteroids. Our patients were mostly on Budesonide or Fluticasone. In our study group 34 (23%) patient's required higher dose of inhaled corticosteroids. Rest of 115(77%) required low or medium dosed steroids.

**Table 7: Requirement of high dose**

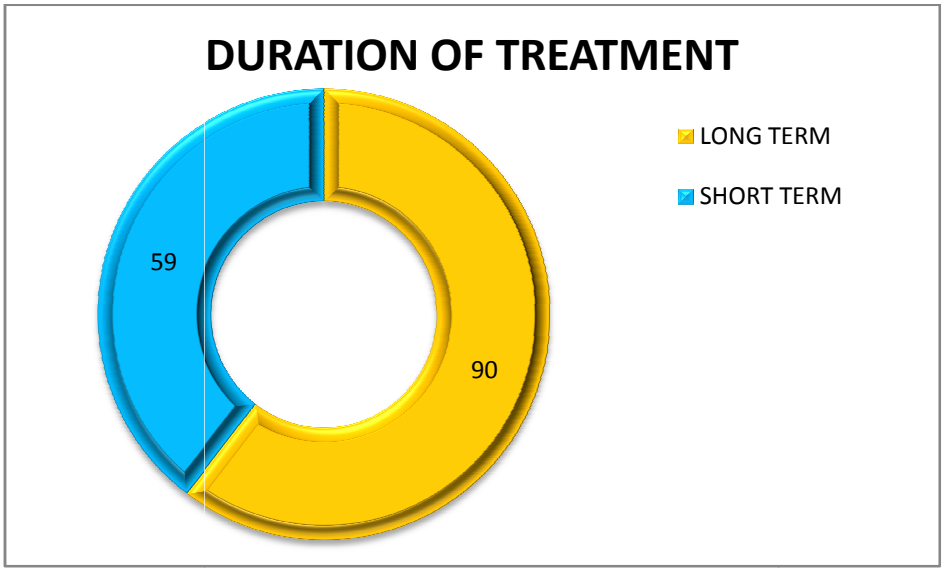


## DURATION OF TREATMENT

**Table 8: Duration of therapy with inhaled steroids**

| DURATION OF TREATMENT | | PERCENTAGE |
|---|---|---|
| LONG TERM(> 3 MONTHS) | **90** | **60.40%** |
| SHORT TERM(< 3 MONTHS) | **59** | **39.60%** |
| TOTAL | **149** | **100%** |

**Figure 8: Duration of therapy with inhaled steroids**



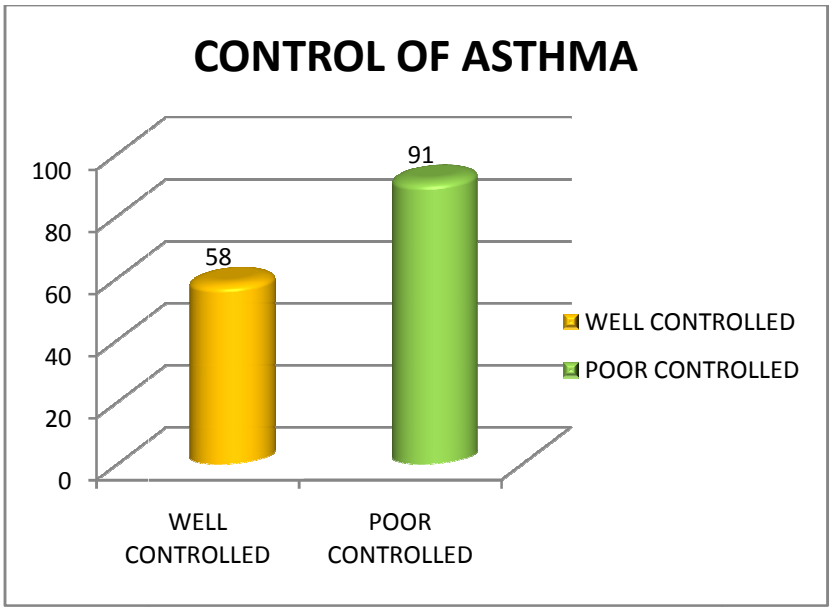

In our study group patients were on steroids ranging from one month to 5 years. Patients were classified based on duration of treatment as long term (more than 3 months) and short term (less than 3 months) based on various studies done in community[4].In our study, higher proportion of patients were on long term treatment, 90(60%) of patients were on long term management of more than 3 months while 59(40%) patients were on short term less than 3 months.

## CONTROL OF ASTHMA

**Table 9: Control of disease**

| CONTROL OF ASTHMA | | PERCENTAGE |
|---|---|---|
| WELL CONTROLLED | **58** | **38.9%** |
| POOR CONTROLLED | **91** | **61.1%** |
| TOTAL | **149** | **100%** |

**Figure 9: Control of disease**

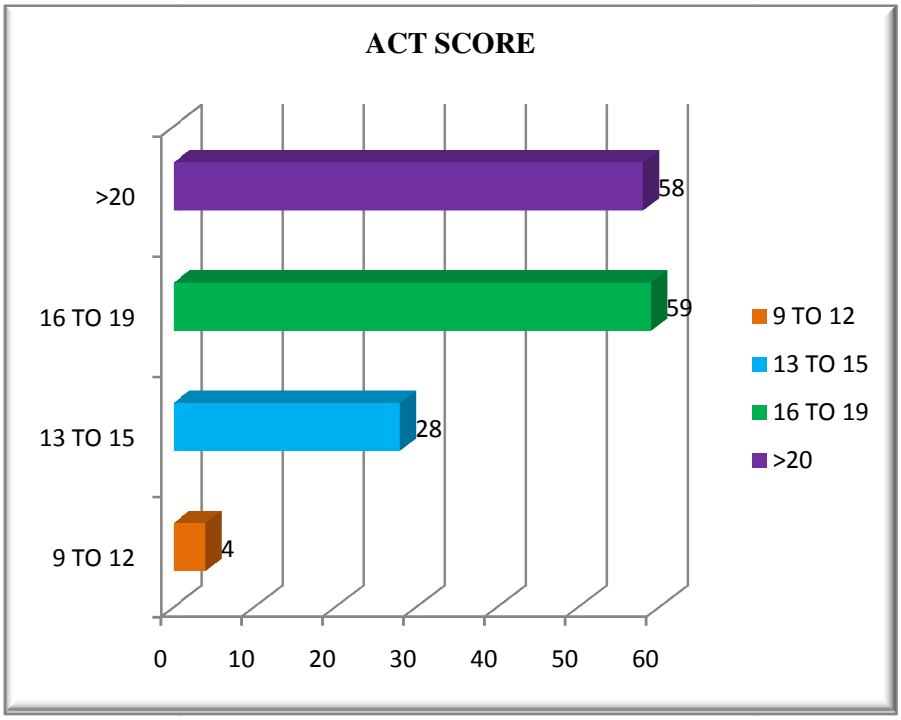



Among 149 patients in our study 58 were well controlled whereas 91 were poorly controlled. They were differentiated into well and poor controlled based on ACT (Asthma Control Test) Questionnaire score

**Table 10: Distribution of ACT score**

| ACT SCORE | | PERCENTAGE |
|---|---|---|
| 9-12 | **4** | **2.7%** |
| 13-15 | **28** | **18.8%** |
| 16-19 | **59** | **39.6%** |
| >20 | **58** | **38.9%** |
| TOTAL | **149** | **100%** |

**Figure 10: Distribution of ACT score**

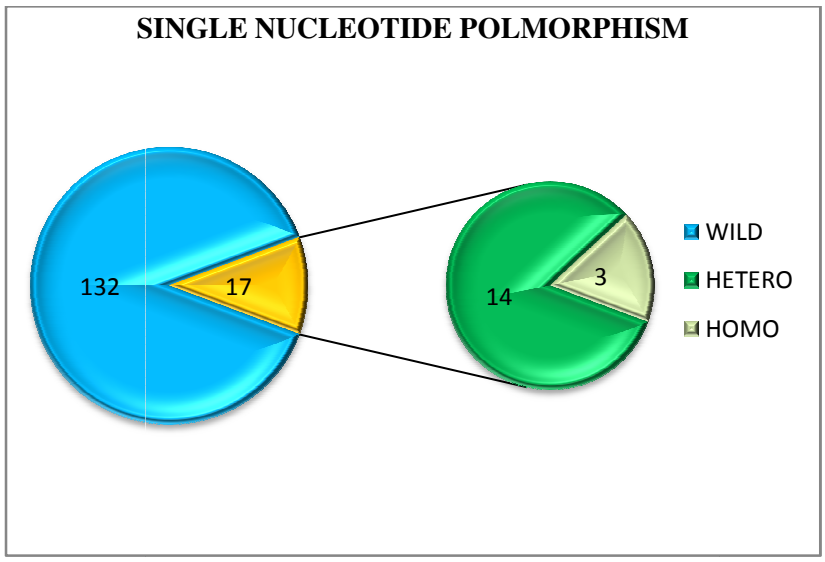


## GRK5 SINGLE NUCLEOTIDE POLYMORPHISM

**Table 11: Prevalence of SNP**

| SNP | TYPE | | TOTAL | PERCENTAGE |
|---|---|---|---|---|
| PRESENT | **HETEROZYGOUS** | **14** | **17** | **11.5%** |
| | **HOMOZYGOUS** | **3** | | |
| ABSENT | **WILD** | | **132** | **88.5%** |
| TOTAL | | | **149** | **100%** |

**Figure 11: Prevalence of SNP**

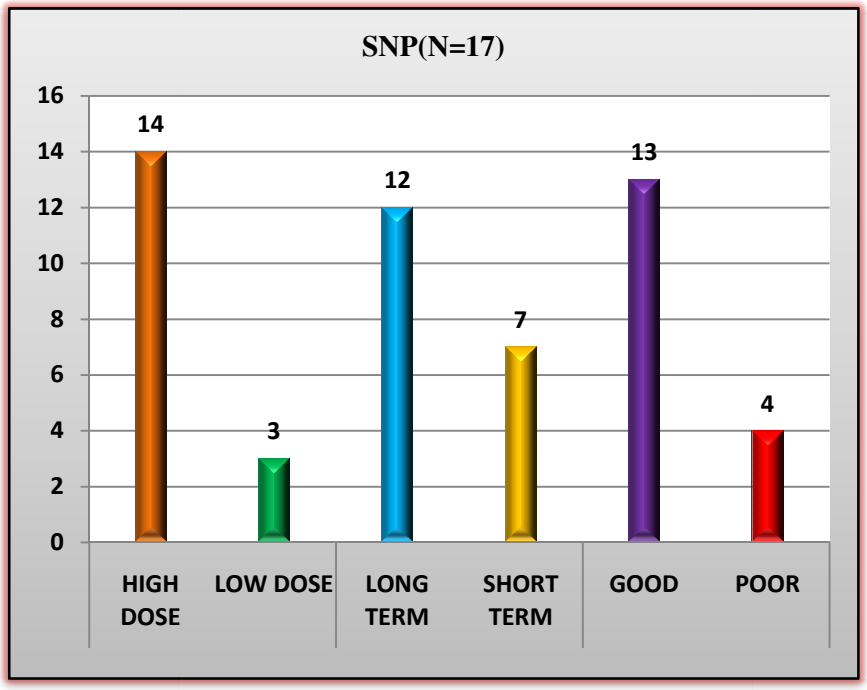



Among 149 patients in our study group 17(11.5%) had SNP, 14 were heterozygous and 3 were homozygous while the rest were wild type.

**Table & Figure 12: Influence of SNP on dose, duration & control**

| SINGLE NUCLEOTIDE POLYMORPHISM (N=17) | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 14 | 17 |
| | LOW DOSE | 3 | |
| DURATION | LONG TERM | 12 | 17 |
| | SHORT TERM | 7 | |
| CONTROL | GOOD | 13 | 17 |
| | POOR | 4 | |

In our study, among the 17 patients with SNP, 14(82.4%) patients required high dose which was statistically significant with P value of <0.001 with Odds ratio of 26.13.Also we observed that 12(70.58%) patients required steroid therapy for long term but was not statistically significant. When we analyzed for association with control of the disease, 13 (76.5%) patients with SNP had good control which was significant with P value of 0.004 and odds ratio of 6.283.

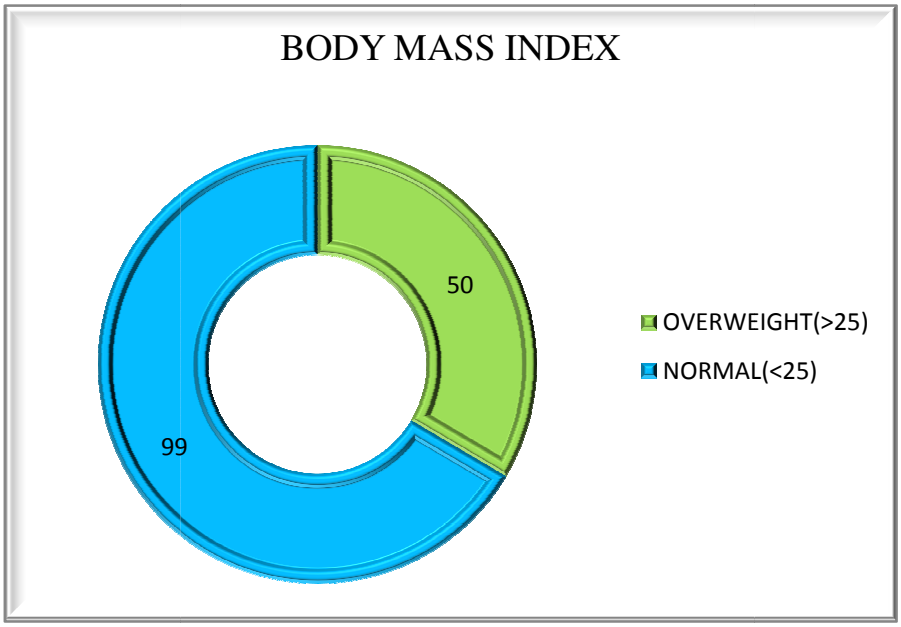


## BODY MASS INDEX

**Table 13: Prevalence of BMI**

| BODY MASS INDEX | | PERCENTAGE |
|---|---|---|
| OVERWEIGHT(>25) | 50 | 33.6% |
| NORMAL(<25) | 99 | 66.4% |
| TOTAL | 149 | 100% |

**Figure 13: Prevalence of BMI**



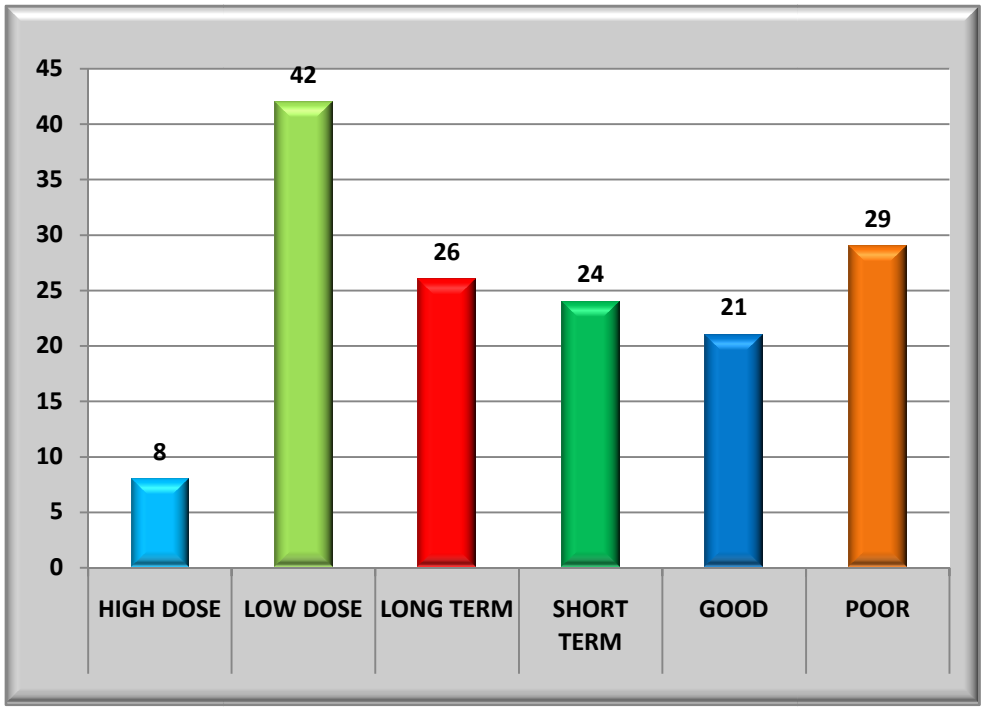

Among 149 patients in our study group 50(33.6%) had their BMI more than 25 kg/$m^2$.While the rest of patients were below 25 kg/$m^2$

**Table 14: Influence of BMI on dose, duration & control**

| OVER WEIGHT(BMI >25 Kg/M$^2$) | | | |
|---|---|---|---|
| **FACTORS** | | **NO OF PATIENTS** | **TOTAL** |
| **DOSE** | **HIGH DOSE** | **8** | **50** |
| | **LOW DOSE** | **42** | |
| **DURATION** | **LONG TERM** | **26** | **50** |
| | **SHORT TERM** | **24** | |
| **CONTROL** | **GOOD** | **21** | **50** |
| | **POOR** | **29** | |

In our study, among 50 patients who were overweight, 8(16%) patients required high dose .Also we observed that 26(52%) patients required steroid therapy for long term. When we analyzed for association with the control of disease, 21(42%) patients with overweight had good control. Influence of higher BMI on all these 3 factors of dose, duration and control were not statistically significant with P value more than 0.05.

**Figure 14: Influence of BMI on dose, duration & control**



## HAZADROUS EXPOSURE

**Table 15.Prevalence of hazardous exposure**

| HAZADROUS EXPOSURE | | PERCENTAGE |
|---|---|---|
| PRESENT | **12** | **8%** |
| ABSENT | **137** | **92%** |
| TOTAL | **149** | **100%** |

Among 149 patients in our study group 12(8%) had hazardous exposure at work like biomass, cotton, tobacco industries while the rest of patients did not have hazardous exposure at work.

**Figure 15.Prevalence of hazardous exposure**

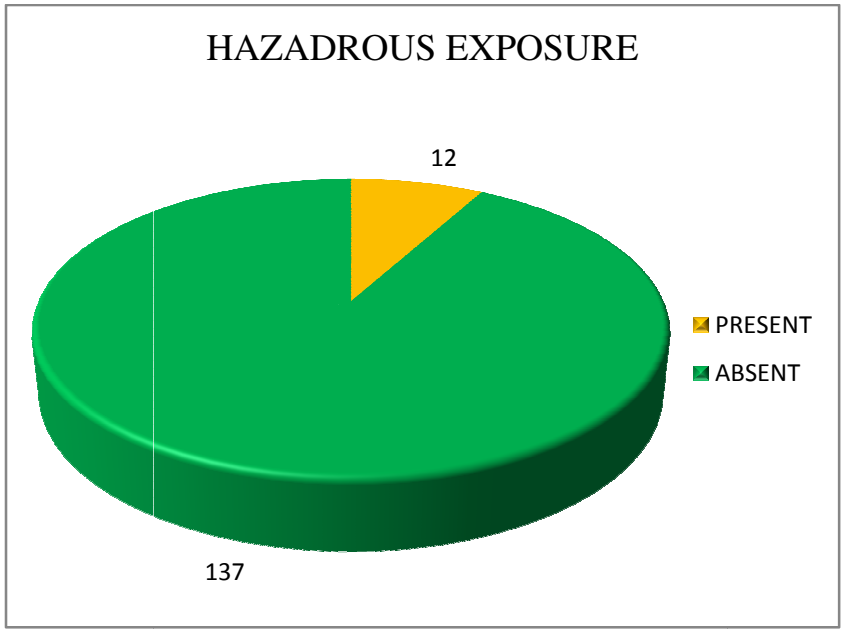
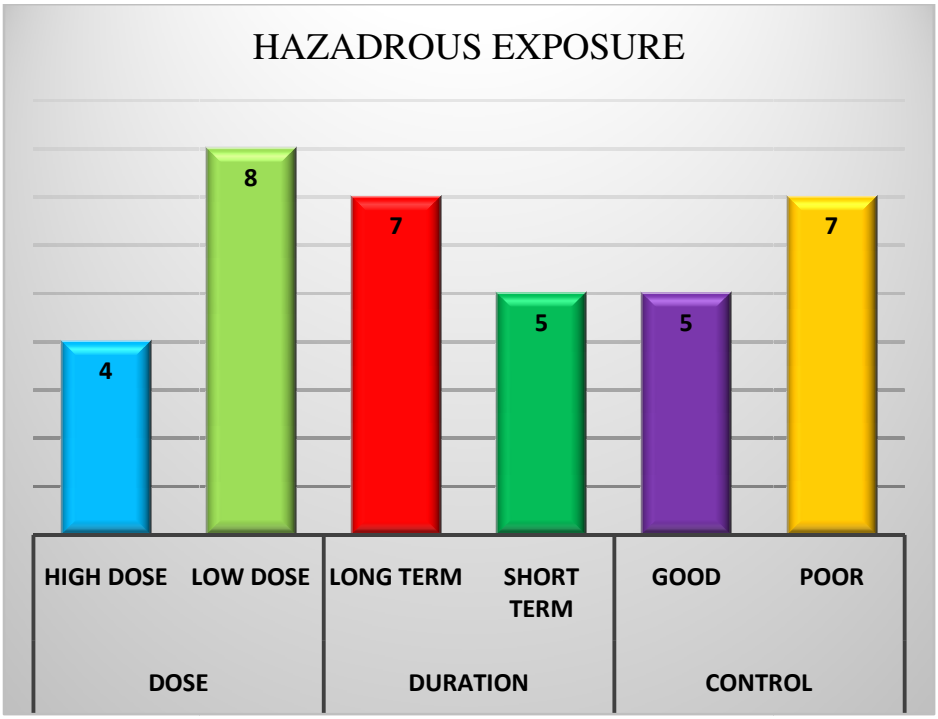


**Table 16: Influence of hazardous exposure on dose, duration & control**

| HAZADROUS EXPOSURE AT WORK | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 4 | 12 |
| | LOW DOSE | 8 | |
| DURATION | LONG TERM | 7 | 12 |
| | SHORT TERM | 5 | |
| CONTROL | GOOD | 5 | 12 |
| | POOR | 7 | |

In our study, among the 12 patients who had history of hazardous exposure, 4(33.33%) patients required high dose. Also we observed that 7(58.33%) patients required steroid therapy for long term. When we analyzed for association with control of disease, 5(41.67%) patient with hazardous exposure had good control. Influence of hazardous exposure on all these 3 factors were not statistically significant with P value more than 0.05.

**Figure 16: Influence of hazardous exposure on dose duration & control**



## SMOKING

**Table 17.Prevalence of smoking**

| SMOKING | | PERCENTAGE |
|---|---|---|
| SMOKERS | **13** | **8.7%** |
| NON SMOKERS | **136** | **91.3%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 13(8.7%) had smoking history among which 2 had history of passive smoking and rest of patients were non-smokers.

**Figure 17.Prevalence of smoking**

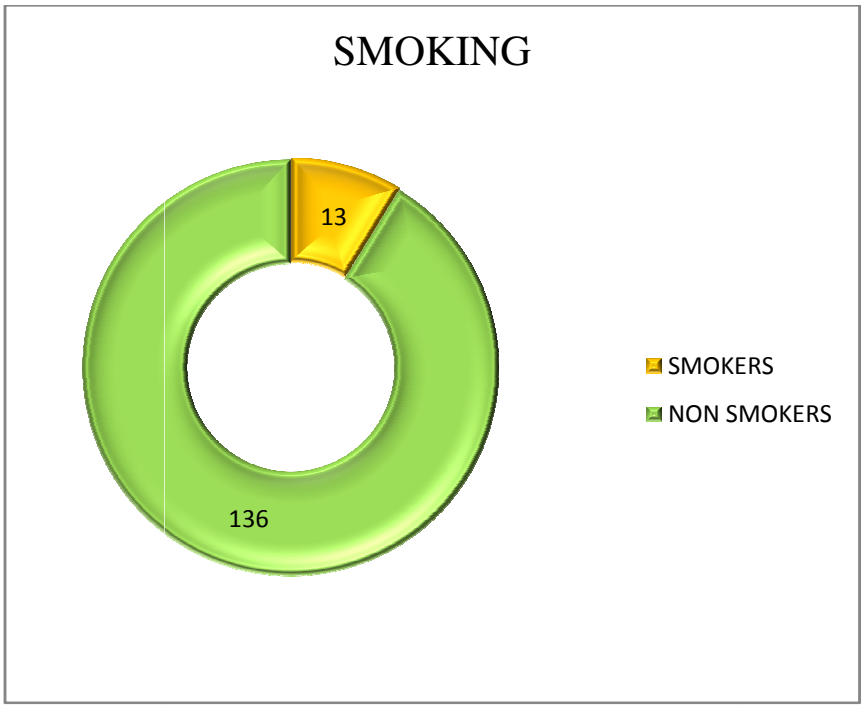
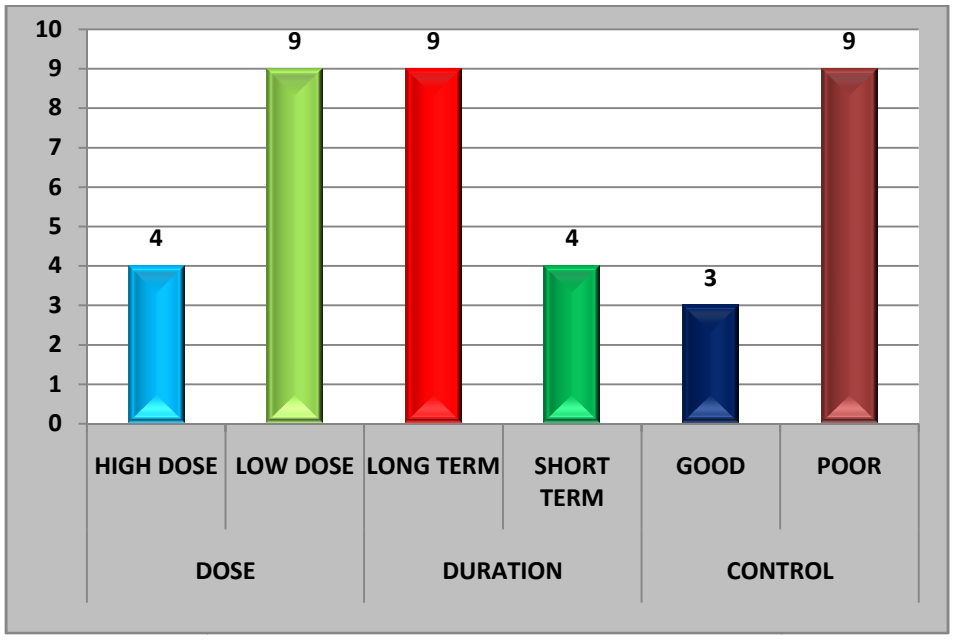


**Table 18: Influence of smoking on dose, duration & control**

| SMOKING | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 4 | 13 |
| | LOW DOSE | 9 | |
| DURATION | LONG TERM | 9 | 13 |
| | SHORT TERM | 4 | |
| CONTROL | GOOD | 3 | 13 |
| | POOR | 9 | |

In our study, among 13 patients who had history of smoking, 4(30.7%) patients required high dose .Also we observed that 9(69.3%) patients required steroid therapy for long term. When we analyzed for association with disease control, 3(23%) patient with smoking had good control. Influence of smoking on all these 3 factors was not statistically significant.

**Figure 18: Influence of smoking on dose, duration & control**



## HISTORY OF ALLERGY OR ATOPY

**Table 19.Prevalence of history of allergy or atopy**

| H/O ALLERGY OR ATOPY | | PERCENTAGE |
|---|---|---|
| PRESENT | **93** | **62.4%** |
| ABSENT | **56** | **37.6%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 93(62.4%) had history to environmental allergens like cold, dust, season, climate and food.

**Figure 19.Prevalence of history of allergy or atopy**

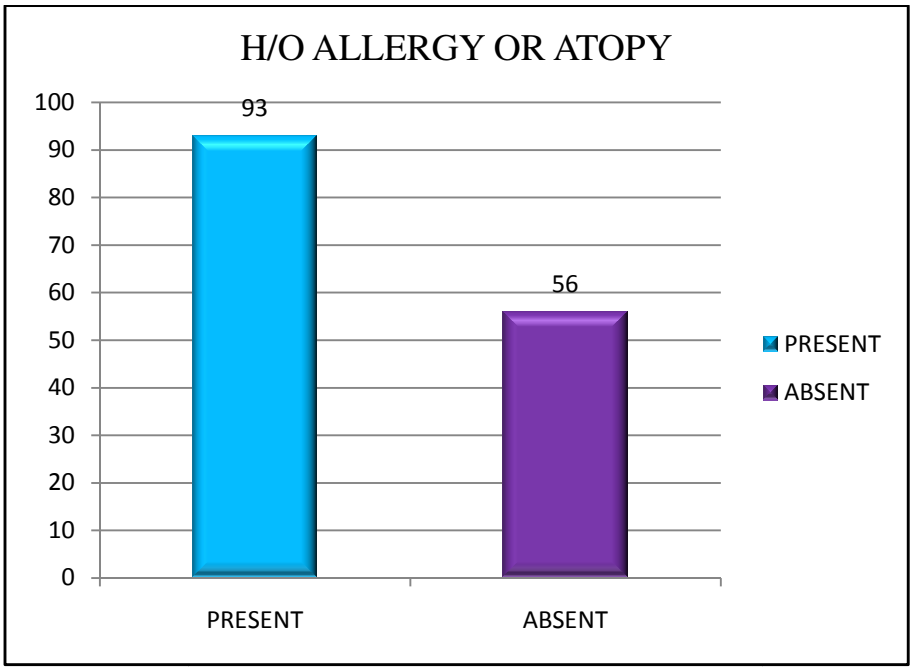
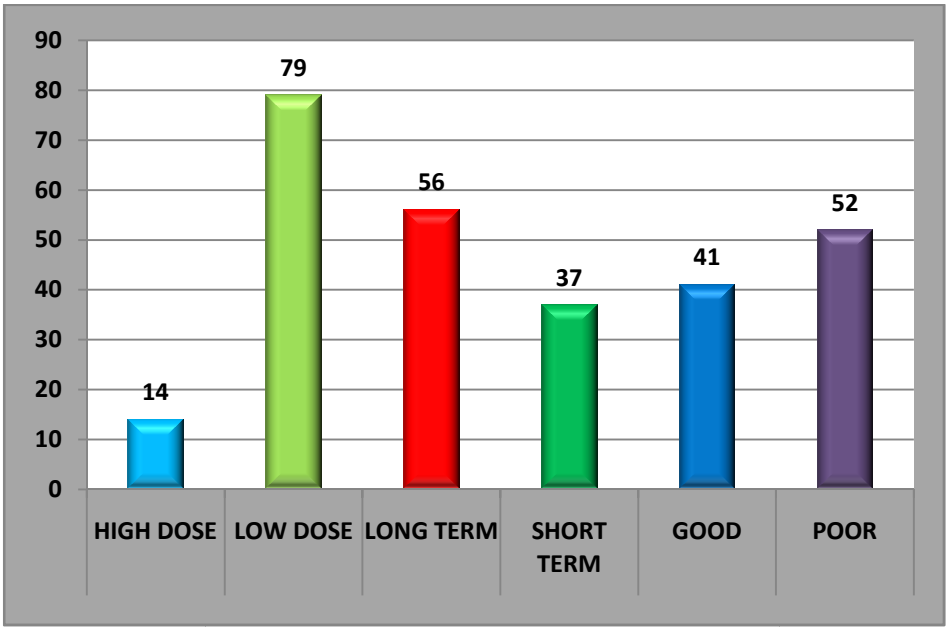


**Table 20: Influence of history of allergy on dose, duration & control**

| HISTORY OF ALLERGY OR ATOPY | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 14 | 93 |
| | LOW DOSE | 79 | |
| DURATION | LONG TERM | 56 | 93 |
| | SHORT TERM | 37 | |
| CONTROL | GOOD | 41 | 93 |
| | POOR | 52 | |

In our study among 93 patients who had history of allergen or atopy, 14(15%) patients required high dose. Also we observed that 56(60%) patients required steroid therapy for long term. When we analyzed for association with control of disease, 41(44%) patients with history of allergy had good control. Influence of history of allergy on duration and control were not statistically significant with P value more than 0.05. But there was no positive correlation between allergy and dose requirement, a P value of 0.004 with OR of 0.319.

**Figure20: Influence of history of allergy on dose, duration & control**



## FAMILY HISTORY

**Table 21.Prevalence of family history**

| FAMILY HISTORY | | PERCENTAGE |
|---|---|---|
| PRESENT | **24** | **16.1%** |
| ABSENT | **125** | **83.9%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 24(16.1%) had family history of bronchial asthma.

**Table 21.Prevalence of FAMILY HISTORY**

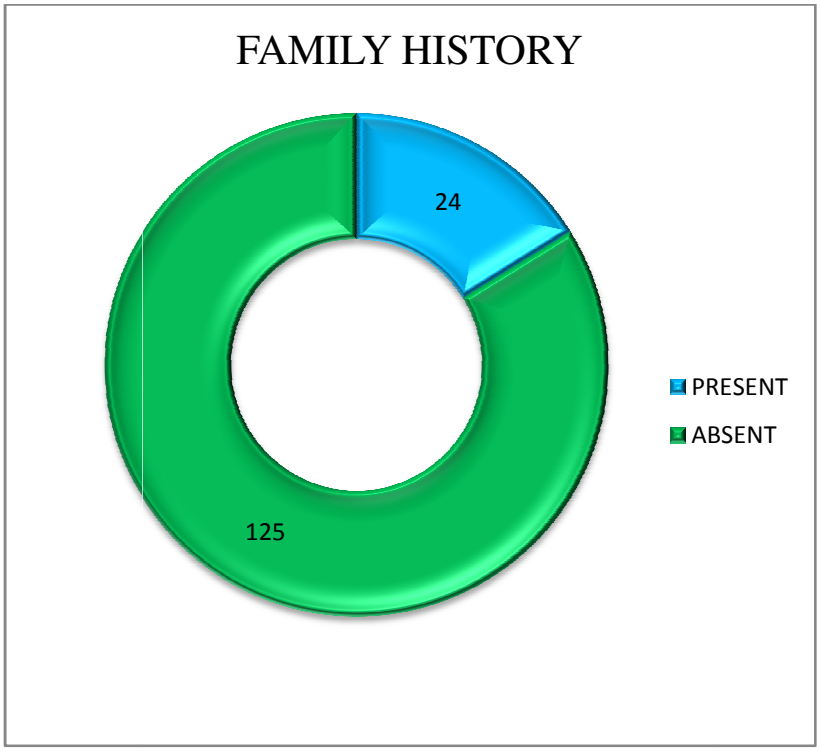
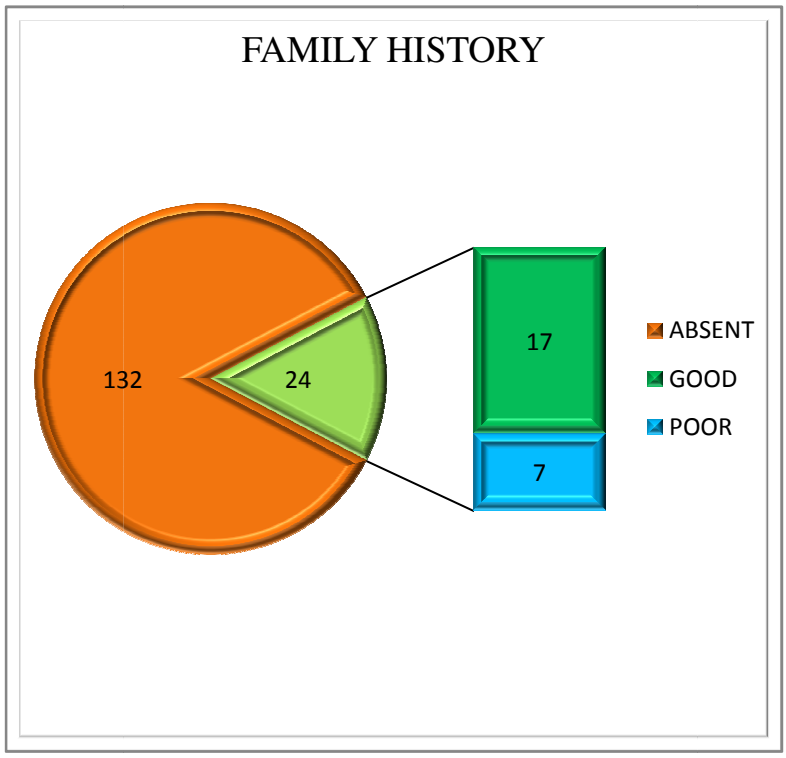


**Table22: Influence of family history on control**

| FAMILY HISTORY | | | |
|---|---|---|---|
| FACTORS | | **NO OF PATIENTS** | **TOTAL** |
| CONTROL | **GOOD** | **7** | **24** |
| | **POOR** | **17** | |

According to our study, positive family history affected the control of disease more than dose requirement and duration. In our study, among 24 patients with family history a lower proportion of patients 7(29%) were under good control compared to those with poor control [17(71%)]. But the difference was not statistically significant.

**Table22: Influence of family history on control**



## RESPIRATORY CO-MORBIDITIES

**Table 23.Prevalence of Respiratory co-morbidities**

| RESPIRATORY CO MORBIDITIES | | PERCENTAGE |
|---|---|---|
| PRESENT | **21** | **14%** |
| ABSENT | **128** | **86%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 21(14%) had associated respiratory co morbidities like respiratory infections, pneumonia, bronchiectasis etc.

**Table 23.Prevalence of Respiratory co morbidities**

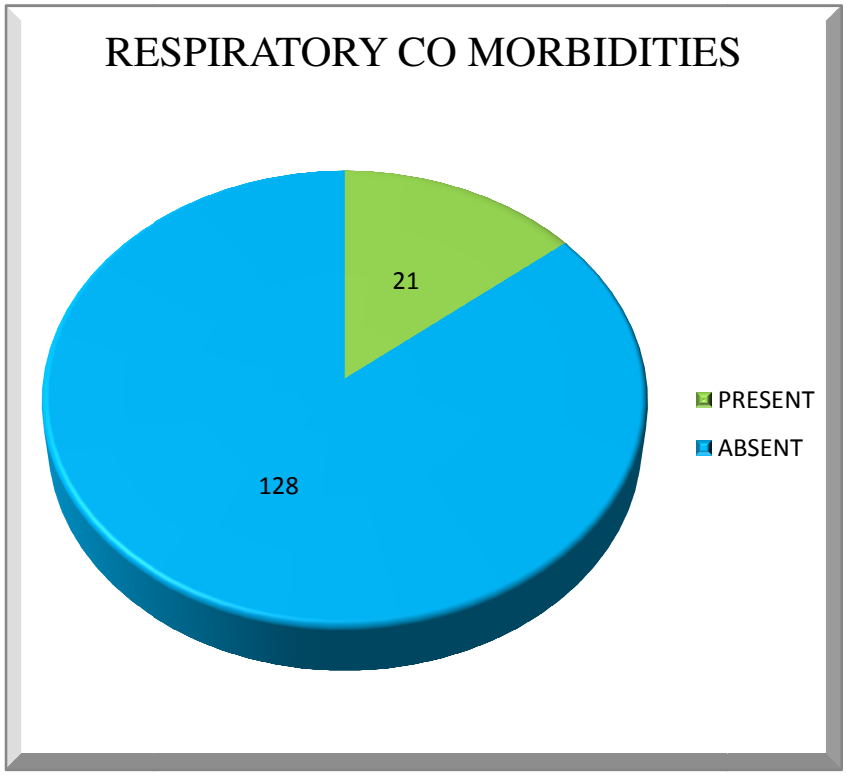
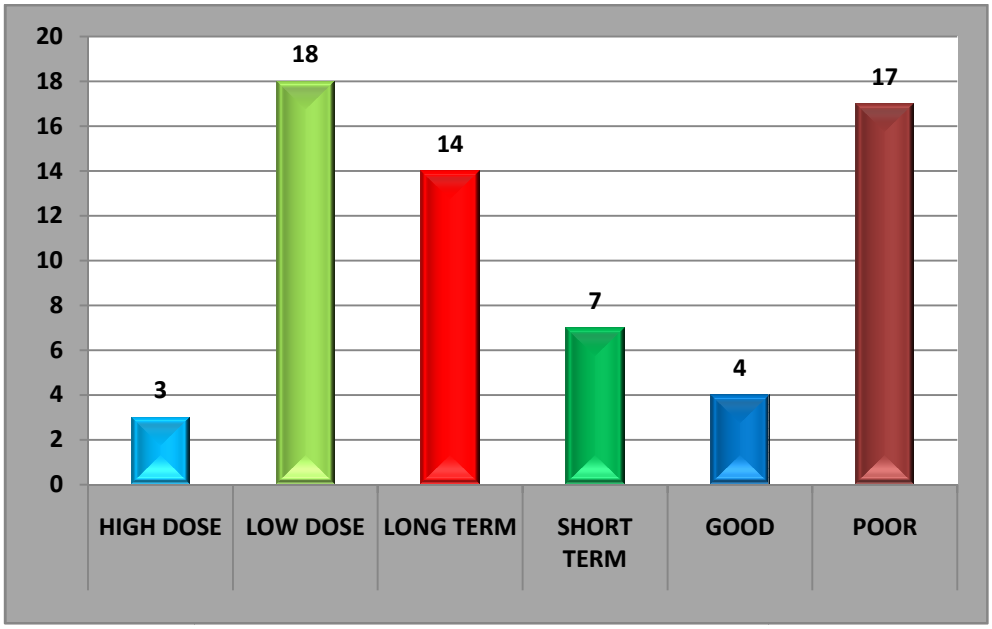


**Table & Figure 24: Influence of respiratory co morbidities on all factors**

| RESPIRATORY CO MORBIDITIES | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 3 | 21 |
| | LOW DOSE | 18 | |
| DURATION | LONG TERM | 14 | 21 |
| | SHORT TERM | 7 | |
| CONTROL | GOOD | 4 | 21 |
| | POOR | 17 | |

In our study, among 21 patients who had history of respiratory co morbidities, 3(15%) patients required high dose. Also we observed that 14(66.66%) patients required steroid therapy for long term. When we analyzed for association with control of disease,4 (19%) patients with history of respiratory co morbidities had good control. Effect on duration and dose requirement was not statistically significant with P value more than 0.05. But patients with respiratory co morbidities had statistically significant effect on poor control of disease with P value of 0.044 with OR of 3.



## ALLERGIC RHINITIS

**Table 25.Prevalence of allergic rhinitis**

| H/O ALLERGIC RHINITIS | | PERCENTAGE |
|---|---|---|
| PRESENT | **55** | **37%** |
| ABSENT | **94** | **63%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 55(37%) had history of allergic rhinitis which is one of most common and important disease associated with asthma.

**Figure 25: Prevalence of allergic rhinitis**

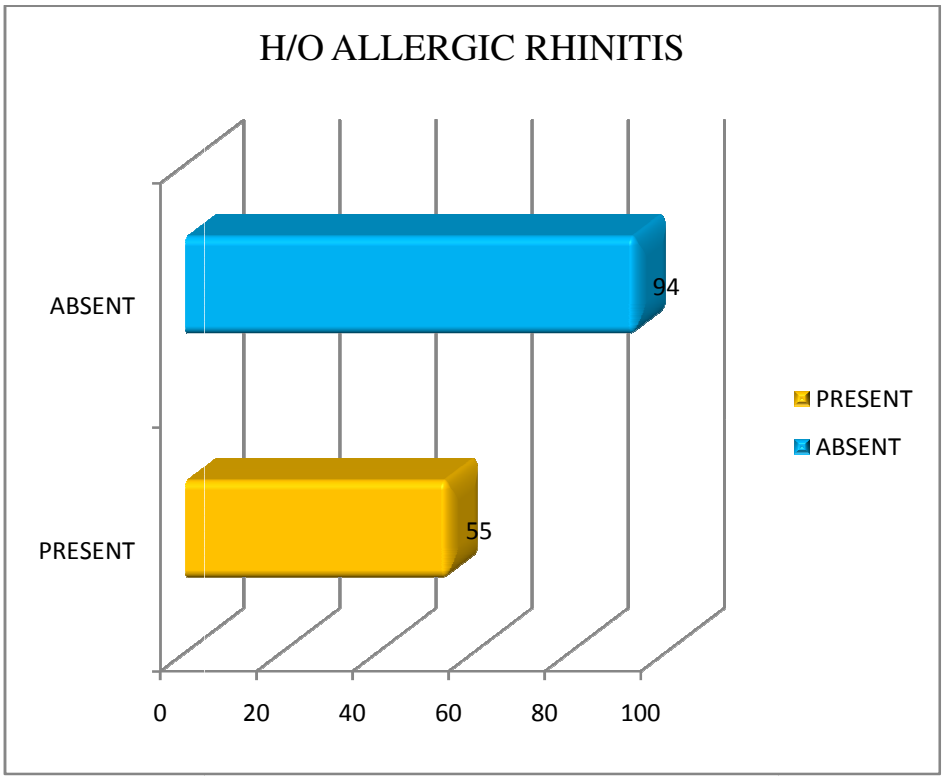
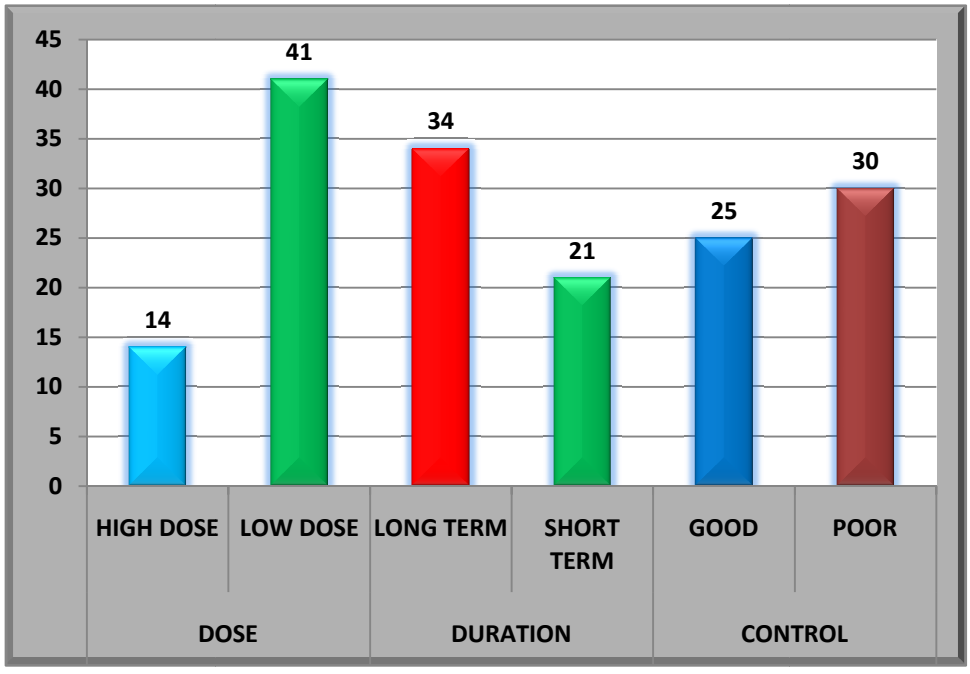


**Table26: Influence of allergic rhinitis on dose, duration & control**

| PRESENCE OF ALLERGIC RHINITIS | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 14 | 55 |
| | LOW DOSE | 41 | |
| DURATION | LONG TERM | 34 | 55 |
| | SHORT TERM | 21 | |
| CONTROL | GOOD | 25 | 55 |
| | POOR | 30 | |

In our study among 55 patients who had history of allergic rhinitis 14(25.5%) patients required high dose. Also we observed that 34(62%) patients required steroid therapy for long term. When we analyzed for association with control of disease, 25(45.5%) patient with allergic rhinitis had good control. Influence of allergic rhinitis on all these 3 factors were not statistically significant with P value more than 0.05.

**Figure 26: Influence of allergic rhinitis on dose, duration & control**



## PREVIOUS HISTORY OF TUBERCULOSIS

**Table 27.Prevalence of previous history of TB**

| PREVIOUS H/O OF TB | | PERCENTAGE |
|---|---|---|
| PRESENT | **8** | **5.4%** |
| ABSENT | **141** | **94.6%** |
| TOTAL | **149** | **100%** |

In our study group among 149 patients 8(5.4%) had previous history of TB and they have treatment for the same in the past.

**Figure 27.Prevalence of previous history of TB**

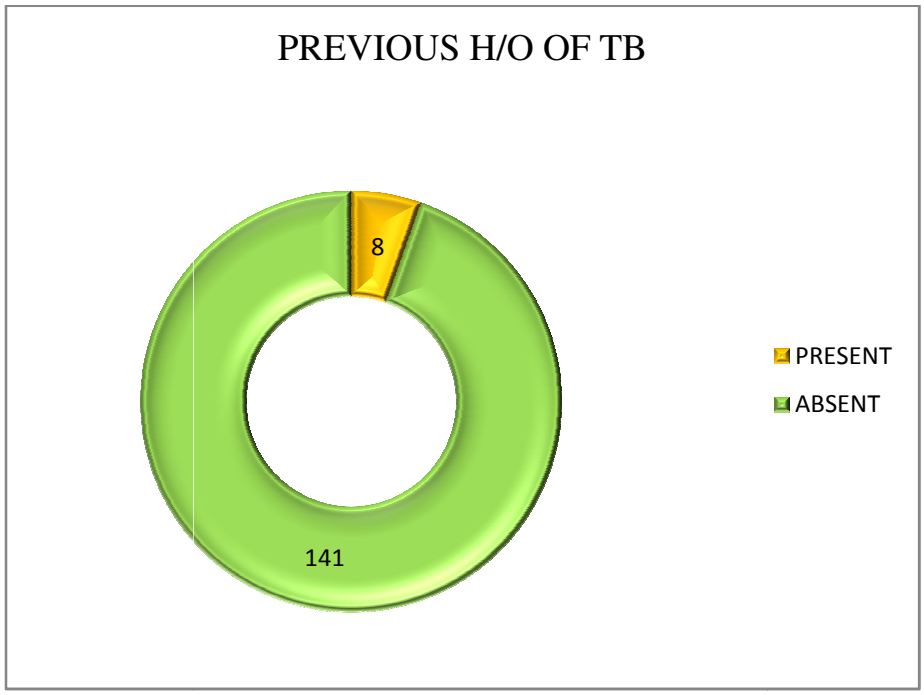
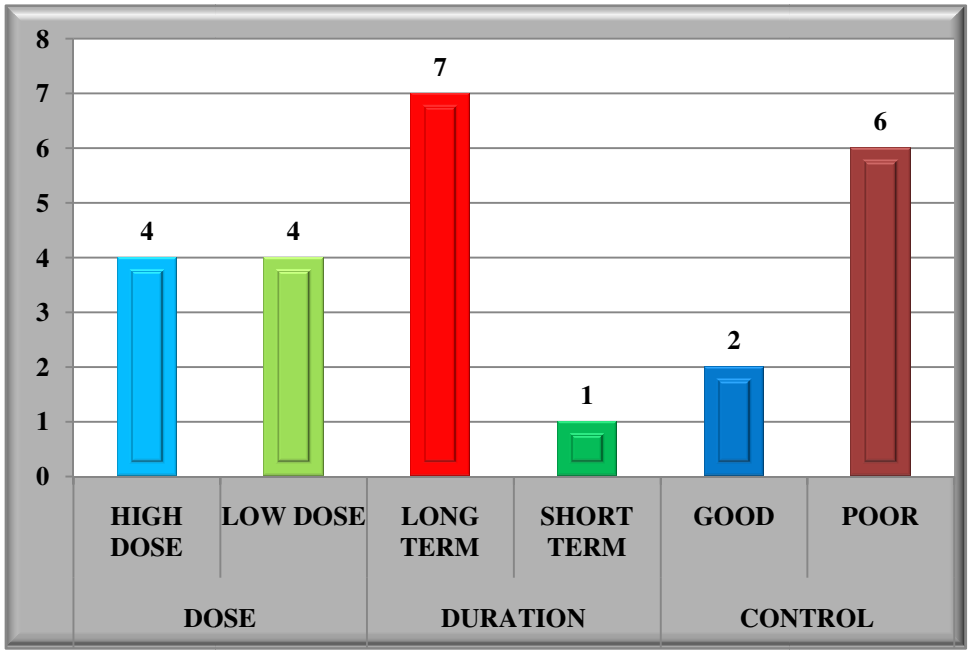


**Table 28: Influence of history of TB on dose, duration & control**

| PREVIOUS HISTORY OF TB | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 4 | 8 |
| | LOW DOSE | 4 | |
| DURATION | LONG TERM | 7 | 8 |
| | SHORT TERM | 1 | |
| CONTROL | GOOD | 2 | 8 |
| | POOR | 6 | |

In our study, among 8 patients who had previous history of TB, 4(50 %) patients required high dose .Also we observed that 7(87.5%) patients required steroid therapy for long term. When we analyzed the association with control of disease, 2(25%) patient with TB had good control. Influence of previous history of TB on all these 3 factors were not statistically significant with P value more than 0.05.

**Figure 28: Influence of history of TB on dose, duration & control**



## OBSTRUCTIVE SLEEP APNEA

**Table 29.Prevalence of OSA**

| OBSTRUCTIVE SLEEP APNEA | | PERCENTAGE |
|---|---|---|
| PRESENT | **13** | **8.7%** |
| ABSENT | **136** | **91.3%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 13(8.7%) had history of obstructive sleep apnea which is an important co morbid factor influencing severity of asthma.

**Figure 29: Prevalence of OSA**

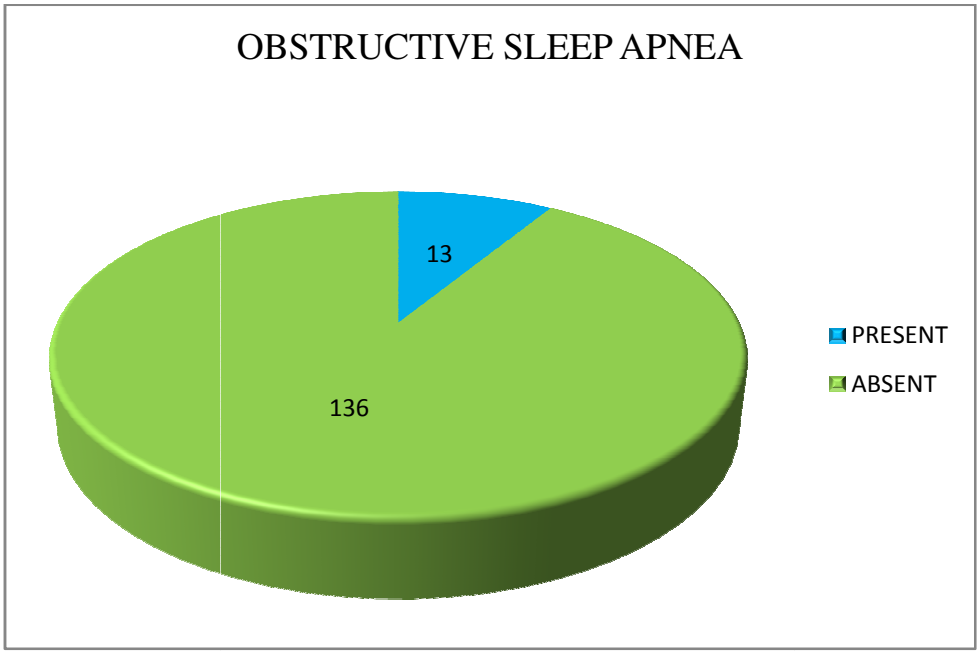
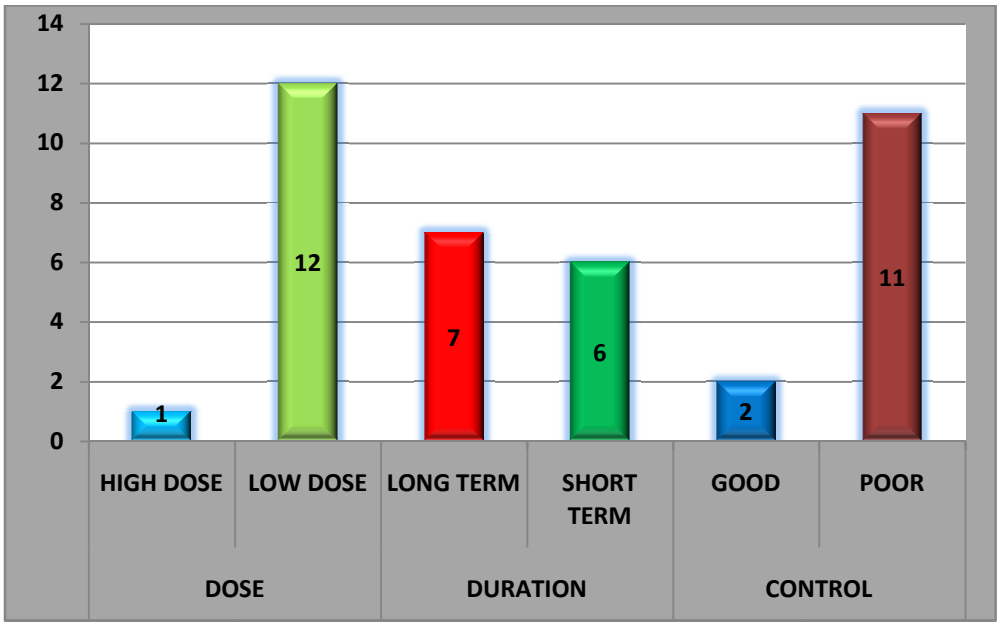


**Table 30: Influence of OSA on dose, duration & control**

| OBSTRUCTIVE SLEEP APNEA | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 1 | 13 |
| | LOW DOSE | 12 | |
| DURATION | LONG TERM | 7 | 13 |
| | SHORT TERM | 6 | |
| CONTROL | GOOD | 2 | 13 |
| | POOR | 11 | |

In our study among 13 patients who had history of OSA, 1(7.6 %) patients required high dose .Also we observed that 7(53.8 %) patients required steroid therapy for long term. When we analyzed for association with control of disease 2(15.5 %) patient with OSA had good control. Influence of OSA on all these 3 factors were not statistically significant with P value more than 0.05.

**Figure 30: Influence of OSA on dose, duration & control**



## GASTRO ESOPHAGEAL REFLUX DISEASE

### Table 31: Prevalence of GERD

| GERD | | PERCENTAGE |
|---|---|---|
| PRESENT | **16** | **10.7%** |
| ABSENT | **133** | **89.3%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 16(10.7%) had GERD symptoms and are presently on treatment.

### Table 31.Prevalence of GERD

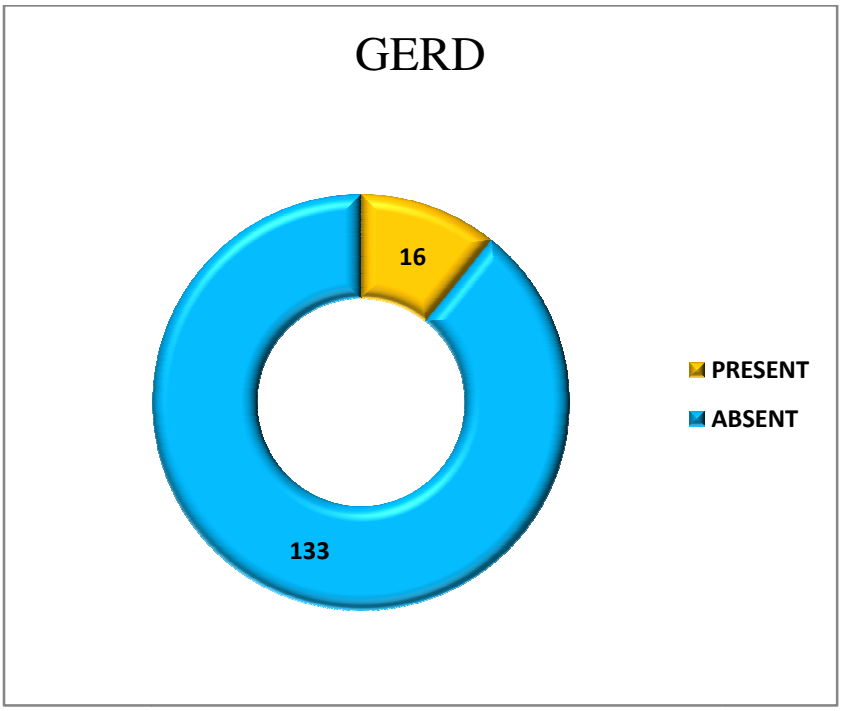
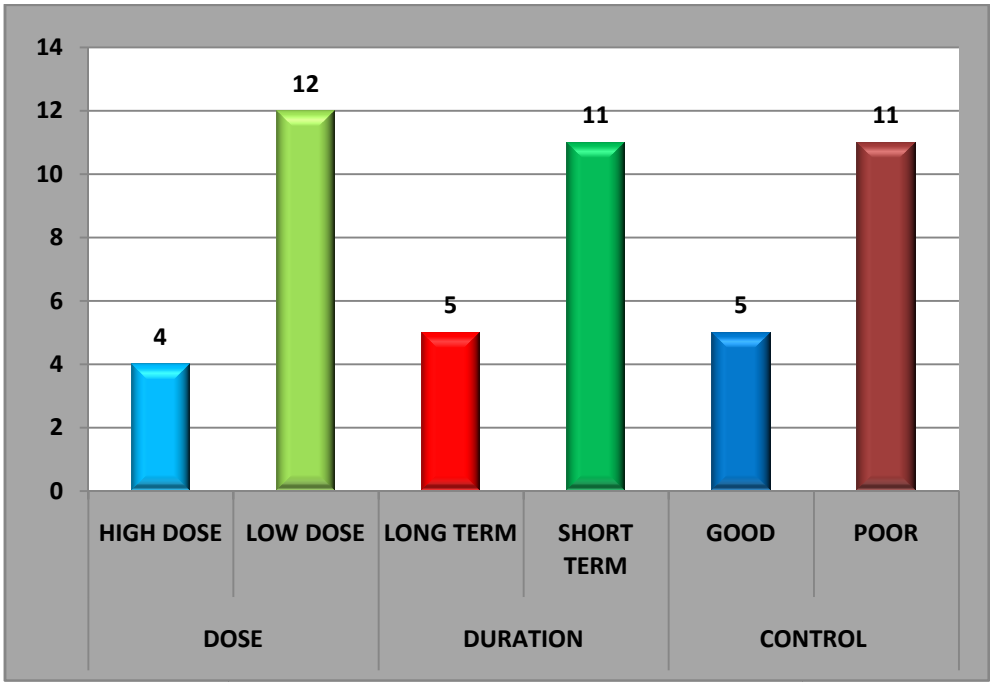


**Table 32: Influence of GERD on dose, duration & control**

| GASTRO ESOPHAGEAL REFLUX DISEASE | | | |
|---|---|---|---|
| FACTORS | | NO OF PATIENTS | TOTAL |
| DOSE | HIGH DOSE | 4 | 16 |
| | LOW DOSE | 12 | |
| DURATION | LONG TERM | 5 | 16 |
| | SHORT TERM | 11 | |
| CONTROL | GOOD | 5 | 16 |
| | POOR | 11 | |

In our study, among 16 patients who had history of hazardous exposure, 4(25%) patients required high dose. Also we observed that 5(31.25%) patients required steroid therapy for long term. When we analyzed for association with control of disease, 5(31.25%) patient with SNP had good control. Influence of SNP on all these 3 factors were not statistically significant with P value more than 0.05.

**Table32: Influence of GERD on dose, duration & control**



## PETS

**Table 33.Prevalence of Pets in home**

| PETS IN HOME | | PERCENTAGE |
|---|---|---|
| PRESENT | **11** | **7.4%** |
| ABSENT | **138** | **82.6%** |
| TOTAL | **149** | **100%** |

In our study among 149 patients 11 (7.4%) had pets in their home which includes dog, cat, cattle, birds etc.

**Table 33.Prevalence of pets in home**

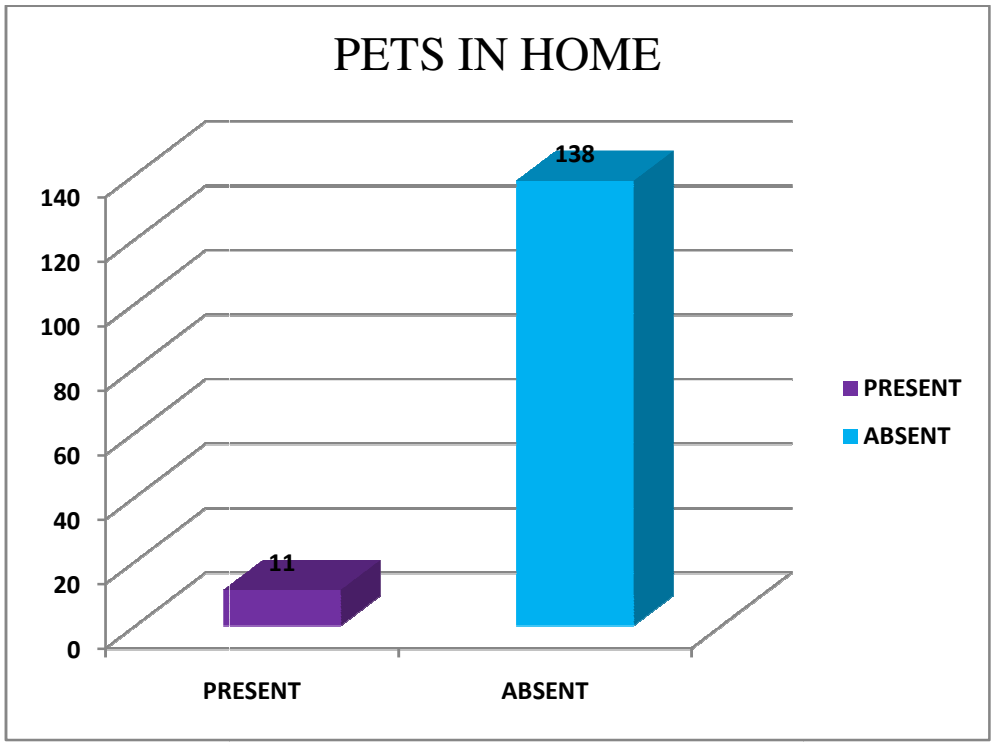
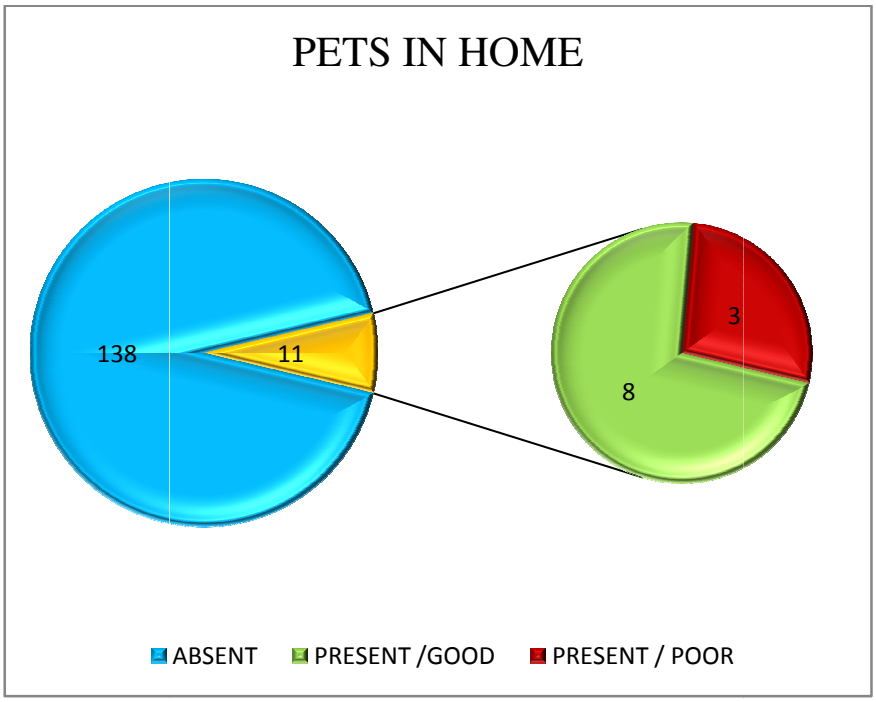


**Table 34: Influence of pets on control of asthma**

| PETS IN HOME | | | |
|---|---|---|---|
| FACTORS | | **NO OF PATIENTS** | **TOTAL** |
| CONTROL | **GOOD** | **3** | **11** |
| | **POOR** | **8** | |

A pet in home had influence mainly on control of asthma more than dose requirement and duration. In our study among 11 patients with exposure to pets at home, 3(27.3%) patients were under good control which is less than patients with poor control [8(72.7%)] with family history. But this was not statistically significant.

.**Table 34: Influence of pets on control of asthma**



# *Discussion*

# DISCUSSION

This cross sectional study was undertaken to evaluate the effect of G-protein receptor kinase-5 (GRK5) polymorphism on the usage of glucocorticoids in the treatment of bronchial asthma. The dose and duration of steroid use and the degree of control of the disease were correlated with the presence of GRK5 polymorphism.

Asthma is a disease whose prevalence keeps increasing every year and steroid dependence in asthma is also on the rise. Even after proper institution of appropriate therapy, increasing numbers of people continue to have poor control of asthma[151].

Our central hypothesis was that an increased dose of steroid and a longer duration of treatment would be required for patients of bronchial asthma with a polymorphism, because this single nucleotide polymorphism (SNP) has been reported to cause endogenous beta adrenergic receptor blockade which might result in poorer overall disease control[152].

The primary inclusion criterion for our study was an asthmatic on maintenance therapy with inhaled corticosteroids. Patients were categorized as receiving low, medium or high dose of inhalational corticosteroid based on the GINA (Global Initiative for Asthma) guidelines for 2015[149]. Based on various studies done in the community[150], patients receiving inhaled steroids for more than 3 months were considered as patients on long term steroid therapy and

those receiving inhaled steroids for less than 3 months were considered as patients on short term steroid therapy.

The total number of patients recruited in our study was 149, among which there were 53 males(35.5%) and 96 females(64.5%). Previous population based studies have shown female sex to be a risk factor for developing asthma[153]. This was also observed in our study where the male: female ratio was 1: 1.8.

In our study we found that there was no significant influence of age over the prevalence of asthma (P=0.954). The number of patients in our study with age more than 40 years (51%) was almost equal to the number of patients with age less than 40 years (49%) and were most commonly in the age group of 41-50 years [39 patients (26%)]. However another multicentric study done in India by Jindal et al[154] showed an increased prevalence of asthma among the age group 15-54 years.

We also studied other factors which are known to influence the mortality and morbidity of asthma, one amongst which is obesity. In our study, 50 patients (33.5%) were overweight with a body mass index (BMI) $\geq$ 25 kg/m$^2$, amongst which, 20 patients were obese with a BMI > 30kg/m$^2$. Our results were similar to various community based studies which showed the prevalence of BMI $\geq$ 25 kg/m$^2$as 30-50% among asthmatics[155].

Exposure to allergens at the work place is another factor which influences the outcome of asthma. In our study 12 patients (8%) had a history of exposure to allergens by working in tobacco industries, cotton mills, etc.

History of known allergy to cold weather, dust and pets is another important risk factor for asthma. In our study more than 79 patients (53%) had a history of allergy to the above mentioned factors. This is similar to various multicentric studies[156] that showed a prevalence of known history of allergy or atopy in 49-63% of patients.

Family history of asthma is another important risk factor which influences the severity of asthma. Our study had 16 patients (10.7%) with a family history of asthma and these results are similar to a study done in the United States in which the prevalence of positive family history in asthmatics ranged from 7-15%[157]

In our study 21 patients (14%) had respiratory co-morbidities like respiratory tract infections, bronchiectasis, pneumonia or previous history of TB, which also influence the morbidity and severity of asthma. Allergic rhinitis is the most common respiratory co-morbidity associated with asthma with a prevalence of 20-40%[158] among asthmatics. The prevalence of allergic rhinitis in our study was similar to previous studies, as 55 patients (37%) were also on treatment for allergic rhinitis.

Obstructive sleep apnea (OSA), diagnosed by a sleep study, and was present in 13 patients (9%). This is almost similar to the prevalence of OSA in asthmatics as reported in literature - 10%– 20%[159].

Gastro esophageal reflux disease (GERD) is another important risk factor for asthma with a known prevalence of more than 20%[160]. In our study the prevalence was slightly less when compared to the literature - 16 patients

(11%) had GERD. This could be due to differences in sample size and patient selection in our study compared to previous studies.

Our primary aim was to evaluate the effect of GRK5 polymorphism in asthmatics on steroid therapy. We studied this gene because of its role in bronchial smooth muscle relaxation. The GRK5 gene controls the expression of a kinase which regulates the G-protein coupled receptors. Phosphorylation induced by GRK5 causes desensitization of the beta adrenergic receptor and enhances endogenous beta receptor blockade. Thus the activity of this kinase may influence disease control and hence the dose requirement and duration of steroid therapy in asthmatics. Agonist promoted $\beta_2$-adrenergic receptor phosphorylation is greater in cells expressing GRK5-Leu41polymorphism, when compared to patients expressing a normal genotype. So GRK5-Leu 41 polymorphism represents a detrimental gain-of-function mutation that causes an enhanced endogenous beta blockade, affecting bronchial smooth muscle relaxation and resulting in poor control of asthma.

In our study, 17 of 149 patients (11.5%) had GRK5 polymorphism. Among those 17 patients, 14 patients were heterozygous, 3 were homozygous and the rest were wild type. It has been found that the prevalence of this polymorphism is variable between different ethnic populations. The GRK5-Leu 41 variant is approximately 10-fold more common in African- Americans[152]. In some races the prevalence of the SNP is in around 2% of the population while in the African Americans it is as high as 36.2%[152]. Our study population had 11.5% of GRK5-Leu41 variant.

Our objective was to study the effect of this SNP on the dosage and duration of treatment with inhaled corticosteroids and also the influence of the SNP on disease control.

Hence we analyzed patients who required high dose inhaled steroids, classified based on the dosage guidelines in the GINA report of 2015 for different inhalational corticosteroids. The majority of patients in our study were receiving Budesonide or Fluticasone. In our study, 34 patients (23%) required high dose inhaled corticosteroids, amongst which 14 patients had SNP and 20 patients had a normal genotype without polymorphism. Among 115 patients (77%) who required low dose inhaled corticosteroids, only 3 patients had the polymorphism.

Interestingly the effect of SNP on requirement of high dose was statistically significant (P=0.000) with an odds ratio of 26.133 with confidence interval of 6.88 to 99.27. Based on this odds ratio, we can state that patients with GRK5 polymorphism have a very high probability of requiring high dose inhaled corticosteroids for adequate control of asthma.

We also assessed the influence of various other factors which may have an effect on the dose requirement of steroids in asthmatics like age, sex, severity of disease, BMI, history of allergy or atopy, respiratory co morbidities like previous history of TB, respiratory infections etc, allergic rhinitis and GERD[161].These factors were included as co-variates and analyzed individually using chi square test.

Among 34 patients requiring high dose inhaled steroids, 8 patients (23.5%) were overweight with BMI more than 25. Only 3 patients (8.8%) had respiratory co-morbidities. History of allergy or atopy was seen in 14 patients (41.1%). In our study, amongst the 34 patients requiring high dose inhaled steroids, 21 patients (61.7%) had poorly controlled disease. 14 patients (41.1%) had allergic rhinitis and 4 patients (11.8%) had GERD.

Except for history of allergy or atopy, the 'P' value of all other factors was not statistically significant. In spite of P value of 0.004 for history of allergy or atopy, the odds ratio was less than 1 (OR=0.319) which indicated that there was no significant positive association between patients with history of allergy and requirement of high dose inhaled steroids.

We also did a multinomial logistic regression analysis with the dependent factor as dose requirement, the factor influencing it as single nucleotide polymorphism and including all other factors considered as co-variates. According to the analysis there was a statistically significant higher dose requirement in patients with SNP.

Another objective was to explore the effect of polymorphism on longer duration of treatment with steroids. In our study group, the duration of steroid use ranged from one month to 5 years. Patients were classified based on duration of treatment as long term steroid use (≥ 3 months) and short term steroid use (≤ 3 months) based on various community-based studies[150]. In our study, higher proportion of patients were on long term treatment – 90 patients

(60%) were on long term steroids while 59 patients (40%) were on short term steroids.

Amongst these 90 patients on long term steroids, 12 patients had SNP. 7 patients in the short term steroids group had SNP. Curiously the effect of SNP on duration of steroid use was not statistically significant with 'p' value of 0.362 and odds ratio of 1.662.

We also analyzed the effect of all previous co-variates on the requirement of steroids for long term therapy[161]. All these factors were also not statistically significant in influencing the duration of treatment. We also did a multinomial logistic regression analysis with the dependent factor as duration of treatment, factor influencing it as single nucleotide polymorphism and including all other factors as co-variates. This did not show any statistically significant association even though the average duration of drug therapy is higher in SNP group(9.65 months) compared to non SNP group(9 months). Based on this analysis we can state that there is no significant effect of SNP on duration of treatment in our study, which implies that the effect of the SNP on the management of asthma is not dependent on the duration of steroid therapy.

Finally we analyzed the effect of SNP on control of asthma, which was measured using the Asthma Control questionnaire, grouping patients having ACT score more than 20 as good control and less than 20 as poor control[162].

In our study group of 149 patients, 91 patients (61%) had poorly controlled asthma and 58 patients (39%) had good disease control. Amongst the 17 patients with SNP, 13 patients (76.5%) had good disease control and the

remaining 4 patients (23.5%) had poorly controlled asthma. This association was also statistically significant with 'p' value of 0.004 and odds ratio of 6.283. This is contrary to the expectation that the endogenous beta adrenergic receptor blockade promoted by this SNP would result in poor disease control.

We also analyzed all the other co-variates which also influence the control of asthma and they were not found to be statistically significant, except for the presence of respiratory co-morbidities. Out of our study group of 149 patients, 21 patients had a history of respiratory co-morbidities, amongst which 17 patients had poorly controlled disease. This association is already well-documented[163]. The association in our study was statistically significant with a P value of 0.044 and an odds ratio of 3.1.

Hence we applied a multivariate logistic regression analysis with dependent factor as control of disease, factor influencing it as single nucleotide polymorphism and including all other factors as co-variates. This analysis showed a statistically significant association for asthmatics with GRK5 polymorphism on inhaled steroids having good disease control. This could be explained from the fact that the SNP has a statistically significant association with high dose requirement of inhaled steroid, which leads to good control of the disease.

Higher dose of glucocorticoids negating the expected effect of the GRK5 polymorphism was also shown in a study done on rats by Judith et al in London[145]where they hypothesized that the impaired relaxation to $\beta_2$-agonists after bronchoconstriction induced by IL-1β, may be due to an increase in the

expression and activity of GRKs, and that this could be reversed by glucocorticoids. The study results were significant in that dexamethasone caused complete inhibition of the increased GRK activity as well as the increased GRK2 and GRK5 protein expression induced by IL-1β.

Similarly in our study, increased expression of GRK5 activity due to polymorphism, resulting in endogenous beta blockade and causing impaired relaxation, may have been reversed by intake of high dose inhaled steroids.

To conclude, based on the analysis from our study, patients with GRK5 polymorphism, receiving inhaled steroids for maintenance therapy of asthma, have more probability of requiring higher dose of inhaled steroid for adequate control of disease. Also administration of high dose steroid can reduce poor disease control due to the effect of the polymorphism. Disease control in patients with polymorphism is not dependent on the duration of inhaled steroid therapy.

# *Conclusion*

# CONCLUSION

1. Asthmatics with GRK5 polymorphism have more probability of requiring high dose steroids for adequate control of disease

2. GRK5 polymorphism does not have a significant effect on the duration of drug therapy.

3. Though patients with polymorphism are expected to have poor control of their asthma, intake of high dose steroids may reverse the effect of the polymorphism and these patients may actually have adequate control of their disease.

4. This study is the first of its type done in India - analyzing the effect of GRK5 polymorphism on steroid therapy in asthmatics.

# *Bibliography*

# REFERENCES


1. http://www.who.int/respiratory/asthma/en/

2. Masoli M, Fabian D, Holt S, Beasley R. Global Initiative for Asthma (GINA) program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59:469–78.

3. Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D, Jindal SK, et al. Asthma Epidemiology Study Group. Prevalence and risk factors for bronchial asthma in Indian adults: a multicentre study. Indian J Chest Dis Allied Sci. 2006;48:13–22.

4. Jindal SK, Aggarwal AN, Gupta D, Agarwal R, Kumar R, Kaur T, et al. Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH) Int J Tuberc Lung Dis. 2012;16:1270–7.

5. Asthma: pathogenesis and novel drugs for treatment J Tod Olin,! Michael E Wechsler

6. Yeatts K, Sly P, Shore S, Weiss S, Martinez F, Geller A, *et al.* A brief targeted review of susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma. *Environ Health Perspect* 2006; *114* : 634-40.

7. Aggarwal AN, Chaudhry K, Chhabra SK, D'Souza GA, Gupta D, Jindal SK, *et al.* Prevalence and risk factors for bronchial asthma in Indian adults: A multicentre study. *Indian J Chest Dis Allied Sci* 2006; *48:* 13-22.

8. Sharma SK, Banga A. Prevalence and risk factors for wheezing in children from rural areas of north India. *Allergy Asthma Proc* 2007; *28:* 647-53.

9. Cookson WOCM. Asthma genetics. *Chest* 2002; *121:*7S-13S.

10. Jaiswal A. Pets can cause asthma. *Chandigarh Tribune*2008; May: 1-2. Available from: h*ttp://www.tribuneindia.com/2008/20080507/cth1.htm*.

11. Phillips KL, An P, Boyd JH, Strunk RC, Casella JF, Barton BA, et al. Major gene effect and additive familiar pattern of inheritance of asthma exist among families of probands with sickle cell anemia and asthma. Am J Hum Biol. 2008; 20:149–53.

12. Liu T, Valdez R, Yoon PW, Crocker D, Moonesinghe R, Khoury MJ. The association between family history of asthma and the prevalence of asthma among US adults: National Health and Nutrition Examination Survey, 1999-2004. Genet Med. 2009; 1:323–28.

13. www.who.int/mediacentre/fact sheets

14. Cochrane MG, Bala MV, Downs KE, Mauskopf J, Ben-Joseph RH. Inhaled corticosteroids for asthma therapy: patient compliance, devices, and inhalation technique. *Chest* 2000; 117:542–50.

15. Bateman ED, Boushey HA, Bousquet J, et al. Can guideline defined asthma control be achieved? The gaining optimal asthma control study. *Am J Respir Crit Care* Med 2004; 170:836–44.

*16.* Harding SM. Gastroesophageal reflux: a potential asthma trigger *Immunol Allergy Clin North Am* 2005; 25:131–48.

17. MacDowell AL, Bacharier LB. Infectious triggers of asthma. *Immunol Allergy Clin North Am* 2005; 25:45–66.

18. Erwin EA, Platts-Mills TA. Allergens. *Immunol Allergy Clin North Am* 2005;25:1–14

19. Tatum AJ, Shapiro GG. The effects of outdoor air pollution and tobacco smoke on asthma. *Immunol Allergy Clin North Am* 2005; 25:15–30.

20. Storms WW. Unmet needs in the treatment of allergic asthma: potential role of novel biologic therapies. *J Manag Care Pharm* 2003; 9:534-43.

21. Mahdi Bijanzadeh, et al .An understanding of the genetic basis of asthma. Indian J Med Res 134, Aug 2011, pp 149-161

22. Salter HH. Asthma: Its Pathology and Treatment, 2nd edition. London: Churchill; 1868.

23. Ashok Shah, Richa Sareen, D Behera , SN Gaur , SK Katiyar. Bronchial Asthma. In NCCP Textbook of Respiratory Medicine. New Delhi: Jaypee; 2011.

24. Schultz WH .Physiological studies in anaphylaxis. I: The reaction of smooth muscle of the guinea-pig sensitized with horse serum. J Pharmacol Exp Ther.1909; 1:566

25. Dunbar WP .The present state of our knowledge of hay fever. J HYg. 1913; 13:105

26. Rackemann FM.A clinical study of one hundred fifty cases of bronchial asthma. Arch Intern Med. 1918; 22:552.

27. Ciba Guest Symposium. Terminology, definitions and classifications of chronic pulmonary emphysema and related conditions. Thorax. 1959; 14:286-99.

28. Guidelines for management of asthma in adults: I-chronic persistent asthma. Br Med J. 1990; 301:651-3.

29. EPR. Expert panel report; Guidelines for the diagnosis and management of asthma (EPR 1991). NIH publication no.91-3642. Bethesda, MD: U.S. Department of Health and Human Services; National Institute of health ;National Heart, lung and Blood Institute; National Asthma Education and Prevention Program;1991 .

30. EPR-2. Expert panel report 2; Guidelines for the diagnosis and management of asthma (EPR-2 1997). NIH publication no.97-4051. Bethesda, MD: U.S. Department of Health and Human Services; National Institute of health ;National Heart, lung and Blood Institute; National Asthma Education and Prevention Program;1997.

31. URL:http//www.ginasthma.org. Global strategy for asthma management and prevention. Updated 2009

32. Murdoch, Jenna R., and Clare M. Lloyd. "Chronic Inflammation and Asthma."Mutation Research 690.1-2 (2010): 24–39.

33. Prue H Hart Regulation of the inflammatory response in asthma by mast cell products Immunology and Cell Biology (2001) 79, 149–153

34. J-F Lauzon-Joset, D Marsolais, A Langlois and E Y Bissonnette Dysregulation of alveolar macrophages unleashes dendritic cell–mediated mechanisms of allergic airway inflammation Mucosal Immunology (2014) 7, 155–164

35. Busse WW, Sedgwick JB Eosinophils in asthma Ann Allergy 1992 Mar;68(3):286-90.

36. Macdowell AL, Peters SP. Neutrophils in asthma. Curr Allergy Asthma Rep 2007 Nov; 7(6):464-8.

37. Kay AB The role of T lymphocytes in asthma. Chem Immunol Allergy 2006;91:59-75.

38.Stephen T. Holgate & Riccardo Polosa Treatment strategies for allergy and asthma Nature Reviews Immunology 8, 218-230 (March 2008)

39. Panettieri RA Jr. Effects of corticosteroids on structural cells in asthma and chronic obstructive pulmonary disease.Proc Am Thorac Soc 2004;1(3):231-4.

40. Barnes PJ, Chung KF, Page CP. Inflammatory mediators of asthma: an update. Pharmacol Rev, 1998a, 50:515–596

41. Barnes PJ. Cytokine networks in asthma and chronic obstructive pulmonary disease. J Clin Invest, 2008a, 118: 3546–3556

42. Palmqvist, C, A J Wardlaw, and P Bradding. "Chemokines and Their Receptors as Potential Targets for the Treatment of Asthma." British Journal of Pharmacology 151.6 (2007): 725–736.

43. Nicholas W. Lukacs, Allison L. Miller and Cory M. Hogaboam Chemokine Receptors in Asthma: Searching for the Correct Immune Targets The Journal of Immunology July 1, 2003vol. 171 no. 1 11-15

44. Sahiner, Umit M et al. "Oxidative Stress in Asthma." The World Allergy Organization journal 4.10 (2011): 151–158.

45. Cho, You Sook, and Hee-Bom Moon. "The Role of Oxidative Stress in the Pathogenesis of Asthma." Allergy, Asthma & Immunology Research 2.3 (2010): 183–187.

46.Bozhena Zoritch Nitric oxide and asthma Archives of Disease in Childhood 1995; 72: 259-262

47. Barnes PJ, Adcock IM Transcription factors and asthma. Eur Respir J 1998 Jul;12(1):221-34.

48. Stephen T. Holgate The sentinel role of the airway epithelium in asthma pathogenesis Immunological Reviews Volume 242, Issue 1, pages 205–219, July 2011

49. Simon G. Roycea, Victor Chenga, Chrishan S. Samuelb, Mimi L.K. Tanga The regulation of fibrosis in airway remodeling in asthma Molecular and Cellular Endocrinology Volume 351, Issue 2, 2012, Pages 167–175

50. Redington AE. Airway fibrosis in asthma: mechanisms, consequences, and potential for therapeutic intervention. Monaldi Arch Chest Dis 2000 Aug; 55(4):317-23.

51. Black JL, Johnson PR Airway smooth muscle in asthma. Respirology 1996 Sep;1(3):153-8.

52. Lee, Chun Geun et al. “Studies of Vascular Endothelial Growth Factor in Asthma and Chronic Obstructive Pulmonary Disease.” Proceedings of the American Thoracic Society 8.6 (2011): 512–515.

53. Evans, Christopher M. et al. “Mucus Hypersecretion in Asthma: Causes and Effects.” Current opinion in pulmonary medicine 15.1 (2009): 4–11.

54. Barnes PJ, Neural mechanisms in asthma. Br Med Bull 1992 Jan;48(1):149-68

55. Bergeron, Céline, Meri K Tulic, and Qutayba Hamid. “Airway Remodelling in Asthma: From Benchside to Clinical Practice.” Canadian Respiratory Journal : Journal of the Canadian Thoracic Society 17.4 (2010): e85–e93

56. O'Byrne PM, Inman MD Airway hyper responsiveness. Chest 2003 Mar;123(3 Suppl):411S-6S.

57. Neil Pearce,Juha Pekkanen,Richard Beasley,How much asthma is really attributable to atopy? *Thorax* 1999; 54:268-272.

58. Cookson WO, Moffatt MF. Genetics of asthma and allergic disease. Hum Mol Genet 2000; 9: 2359–2364.

59. Barnes KC. Evidence for common genetic elements in allergic disease. J Allergy Clin Immunol 2000; 106: S192–S200.

60. Donald K. Blumenthal, James C.Garrison. Pharmacodynamics:Molecular mechanisms of drug action. In: Laurence L.Brunton. The pharmacological basis of therapeutics. 12. China: McGraw-Hill; 2011

61. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. Science, 2000, 289:739–745

62. Gilman AG. G proteins: Transducers of receptor-generated signals. Annu Rev Biochem, 1987, 56:615–649

63. Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: Regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem, 2000, 69:795–827

64. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. Annu Rev Physiol, 2007, 69:451–482

65. Sebastian l. Johnston et al The relationship between upper respiratory infedions and hospital admissions for asthma: a nme-trend analysis Am J Respir Crit Care Med 1996;154:654-60

66. Riccioni G, Barbara M, Bucciarelli T, di Ilio C, D'Orazio N. Antioxidant vitamin supplementation in asthma. Ann Clin Lab Sci 2007 Winter;37(1):96-101.

67. Mickleborough TD, Ionescu AA, Rundell KW Omega-3 Fatty acids and airway hyperresponsiveness in asthma J Altern Complement Med 2004 Dec;10(6):1067-75..

68. Mario Castro et al, Effect of Vitamin $D_3$ on Asthma Treatment Failures in Adults with Symptomatic Asthma and Lower Vitamin D Levels JAMA. 2014; 311(20):2083-2091

69. M. Brauer, B. Brunekreef Air pollution and development of asthma, allergy and infections in a birth cohort ERJ May 1, 2007 vol. 29 no. 5879-888

70. Van der Zee SC,Hoek G , Bosen HM , et al . . Acute effects of urban air pollution on respiratory helath of children and without chronic respiratory symptoms. Occup Environ Med 1999; 56:802 -12

71. Buchdahl R, Parker A, Stebbings T,Babiker A.Association between air pollution and acutechildhood wheezy episodes:prospective observational study . BMJ 1996 ;312:661-5

72. Kesten S,Szalai J.Dzyngel B . Air quality and the frequency of emergency room visits for asthma. Ann Allergy Asthma Immunol 1995; 74:269-73

73. Catarina Almqvist, Magnus Wickman, Luca Perfetti, Niklas Berglind, Anne Renström, Marie Hedrén, Kjell Larsson, Gunilla Hedlin, And Per Malmberg "Worsening of Asthma in Children Allergic to Cats, after Indirect Exposure to Cat at School", American Journal of Respiratory and Critical Care Medicine, Vol. 163, No. 3 (2001), pp. 694-698.

74. Cristina Elisabetta Mapp Agents, Old And New, Causing Occupational Asthma Occup Environ Med. 2001 May; 58(5): 354–360.

75. Green BJ, Beezhold DH. Industrial Fungal Enzymes: An Occupational Allergen Perspective. Journal of Allergy. 2011; 2011:682574

76. Delgado J, Barranco P, Quirce S. Obesity and asthma. J Investig Allergol Clin Immunol 2008; 18(6):420-5.

77. Laerum BN et al Young maternal age at delivery is associated with asthma in adult offspring. . Respir Med 2007 Jul;101(7):1431-8. Epub 2007 Mar 12

78. Yousuke Takemura et al Relation between Breastfeeding and the Prevalence of Asthma -The Tokorozawa Childhood Asthma and Pollinosis Study Am. J. Epidemiol (2001) 154 (2):115-119.

79. Brooks AM, Byrd RS, Weitzman M, Auinger P, McBride JT. Impact of low birth weight on early childhood asthma in the United States. Arch Pediatr Adolesc Med 2001 Mar;155(3):401-6

80 Barnes PJ Intrinsic asthma: not so different from allergic asthma but driven by superantigens? Clin Exp Allergy 2009 Aug;39(8):1145-51.

81. Friedlander SL, Busse WW. The role of rhinovirus in asthma exacerbations. J Allergy Clin Immunol 2005 Aug;116(2):267-73.

82. Wu P, Hartert TV. Evidence for a causal relationship between respiratory syncytial virus infection and asthma. Expert review of anti-infective therapy. 2011;9(9):731-745.

83. Simon D Message and Sebastian L Johnston Viruses in asthma British Medical Bulletin 2002;61: 29–43

84. Covar RA, Macomber BA, Szefler SJ Medications as asthma triggers. Immunol Allergy Clin North Am 2005 Feb;25(1):169-90.

85. E.R. McFadden, Jr., and Ileen A. Gilbert Exercise-Induced Asthma,N Engl J Med 1994; 330:1362-1367

86. K. Larsson, G. Tornling, D. Gavhed, C. Müller-Suur, L. Palmberg Inhalation of cold air increases the number of inflammatory cells in the lungs in healthy subjects Eur Respir J 1998; 12: 825–830

87. NM Wilson, PJ Barnes, H Vickers, M Silverman Hyperventilation-induced asthma: evidence for two mechanisms Thorax 1982;37:657-662

88. Liangas G, Yates DH, Wu D, Henry RL, Thomas PS Laughter-associated asthma J Asthma. 2004 Apr;41(2):217-21.

89. P N G Med J. 1992 Sep;35(3):186-90. Precipitation of asthma attacks in Melanesian adults by sodium metabisulphite. Timberlake CM, Toun AK, Hudson BJ.

90. Br Med J (Clin Res Ed). 1984 Jan 28; 288(6413): 314–315. Hyperthyroidism and acute bronchial asthma. W J Fitzpatrick, P S Foreman, E J Porter, and A G Beckett

91. Oppedal RJ, Khan DA, Brown ES. Hypothyroidism in Patients with Asthma and Major Depressive Disorder. Primary Care Companion to the Journal of Clinical Psychiatry. 2007;9(6):467-468.

92. Bowrey DJ, Peters JH, DeMeester TR. Gastroesophageal Reflux Disease in Asthma: Effects of Medical and Surgical Antireflux Therapy on Asthma Control.Annals of Surgery. 2000;231(2):161-172.

93. Gaude, Gajanan S. “Pulmonary Manifestations of Gastroesophageal Reflux Disease.” Annals of Thoracic Medicine 4.3 (2009): 115–123.

94. Chen E, Miller GE. Stress and Inflammation in Exacerbations of Asthma. Brain, behavior, and immunity. 2007;21(8):993-999.

95. Jerry A. Krishnan et al Asthma outcomes: Symptoms J Allergy Clin Immunol Volume 129, Number 3 S124-S135

96. Beasley, Richard Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC The Lancet , Volume 351 , Issue 9111 , 1225 – 1232

97. Peat JK, Woolcock AJ, Cullen K Rate of decline of lung function in subjects with asthma.European Journal of Respiratory Diseases [1987, 70(3):171-179]

98. R. Pellegrino et al Interpretative strategies for lung function tests Eur Respir J 2005; 26: 948–968

99. Michael P DeLorme' , Owen R Moss Pulmonary function assessment by whole-body plethysmography in restrained versus unrestrained mice Journal of Pharmacological and Toxicological Methods Volume 47, Issue 1, 2002, 1–10

100. V. Backeret al Sensitivity and specificity of the histamine challenge test for the diagnosis of asthma in an unselected sample of children and adolescents Eur Respir J 1991, 4, 1093-1100

101. K. Aas, U. Lundkvist The radioallergosorbent test with a purified allergen from codfish Clinical & Experimental Allergy Volume 3, Issue 3 September 1973 Pages 255–261

102. F. Paganin, V. Trussard, E. Seneterre, P. Chanez, J. Giron, P. Godard, J. P. Sénac, F. B. Michel, and J. Bousquet "Chest Radiography and High Resolution Computed Tomography of the Lungs in Asthma", American Review of Respiratory Disease, Vol. 146, No. 4 (1992), pp. 1084-1087.

103. D J Hendrick, R J Davies, M F D'Souza, J Pepys An analysis of skin prick test reactions in 656 asthmatic patients. Thorax 1975; 30:2-8

104. P. J. Barnes and S. A. Kharitonov.Exhaled nitric oxide: a new lung function test. Thorax. 1996 Mar; 51(3): 233–237.

105. William Kelly , Christine A Sorkness . Asthma. In: Joseph T Dipiro , Robert L Talbert .Pharmacotheraphy, A Pathophysiologic approach . 7th edition . China : McGraw Hill ;2008

106. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids: An update. Am J Respir Crit Care Med, **1998**, 157: S1–S53.

107. Clark AR. MAP kinase phosphatase 1: A novel mediator of biological effects of glucocorticoids? *J Endocrinol*, **2003**, *178*:5–12.

108. Goodman and Gillman testbook of pharmacology,12th edition,Mcgraw Hill.Pulmonary Pharmacology,Peter J Barnes 983-1004

109. Erin EM, Zacharasiewicz AS, Nicholson GC, et al. Rapid anti-Inflammatory effect of inhaled ciclesonide in asthma: A randomised, placebo-controlled study. *Chest*, **2008**, *134*: 740–745.

110.Goodman and Gillman textbook of pharmacology,12th edition,Mcgraw Hill.Pulmonary Pharmacology,Peter J Barnes 983-1004

111. Barnes PJ. Scientific rationale for combination inhalers with a long-acting β2-agonists and corticosteroids. *Eur Respir J*, **2002**, *19*:182–191.

112. Giembycz MA, Kaur M, Leigh R, Newton R. A Holy Grail of asthma management: Toward understanding how long-acting beta(2)-adrenoceptor agonists enhance the clinical efficacy of inhaled corticosteroids. *Br J Pharmacol*, 2008, *153*:1090–1104.

113. Usmani OS, Ito K, Maneechotesuwan K, et al. Glucocorticoid receptor nuclear translocation in airway cells following inhaled combination therapy. Am J Respir Crit Care Med, 2005, 172:704–712.

114. Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids: An update. Am J Respir Crit Care Med, 1998b, 157: S1–S53.

115. Adams NP, Bestall JC, Jones P, et al. Fluticasone at different doses for chronic asthma in adults and children. Cochrane Database Syst Rev, 2008, CD003534

116. Hawkins G, McMahon AD, Twaddle S, et al. Stepping down inhaled corticosteroids in asthma: Randomised controlled trial. BMJ, 2003, 326:1115.

117. Ruffin RE, McIntyre E, Crockett AJ, Zielonka K, Alpers JH. Combination bronchodilator therapy in asthma. J Allergy Clin Immunol 1982 Jan;69(1 Pt 1):60-5.

118. Morgan SJ, Deshpande DA, Tiegs BC, et al. β-Agonist-mediated Relaxation of Airway Smooth Muscle Is Protein Kinase A-dependent. The Journal of Biological Chemistry. 2014;289(33):23065-23074.

119. Tian JW, Chen JW, Chen R, Chen X. Tiotropium versus placebo for inadequately controlled asthma: a meta-analysis. . Respir Care 2014 May;59(5):654-66

120. Ohta K et al Japanese Guideline for Adult Asthma 2014. Allergol Int 2014 Sep;63(3):293-333.

121. Federica Novelli, Laura Malagrinò, Federico L Dente & Pierluigi Paggiaro Efficacy of anticholinergic drugs in asthma. Expert Review of Respiratory Medicine,6(33) 2012; 309-319

122. Ducharme FM, Lasserson TJ, Cates CJ. Long-acting beta2-agonists versus anti-leukotrienes as add-on therapy to inhaled corticosteroids for chronic asthma. Cochrane Database Syst Rev, 2006, CD003137.

123. Robinson DS, Campbell DA, Barnes, PJ. Addition of an anti-leukotriene to therapy in chronic severe asthma in a clinic setting: A double-blind, randomised,placebo-controlled study. Lancet, 2001, 357:2007–2011.

124. Padullés Zamora N et al Retrospective analysis of omalizumab in patients with severe allergic asthma. Farm Hosp 2013 Sep-Oct;37(5):399-405.

125. Cox L, Platts-Mills TA, Finegold I, et al. American Academy of Allergy, Asthma & Immunolog American College of Allergy, Asthma and Immunology Joint Task Force Report on omalizumab-associated anaphylaxis. J Allergy Clin Immunol, 2007, 120:1373–1377.

126. Avila PC. Does anti-IgE therapy help in asthma? Efficacy and controversies. Annu Rev Med, 2007, 58:185–203.

127. Maes T, Joos GF, Brusselle GG. Targeting interleukin-4 in asthma: lost in translation? Am J Respir Cell Mol Biol 2012; 47: 261–70.

128. Borish LC, Nelson HS, Corren J, et al. Effi cacy of soluble IL-4 receptor for the treatment of adults with asthma. J Allergy Clin Immunol 2001; 107: 963–70.

129. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Eff ect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. Lancet 2007; 370: 1422–31.

130. Corren J, Busse W, Meltzer EO, et al. A randomized, controlled, phase 2 study of AMG 317, an IL-4R alpha antagonist, in patients with asthma. Am J Respir Crit Care Med 2010; 181: 788–96.

131. Flood-Page P, Swenson C, Faiferman I, et al. A study to evaluate safety and effi cacy of mepolizumab in patients with moderate persistent asthma. Am J Respir Crit Care Med 2007; 176: 1062–71.

132. Castro M, Mathur S, Hargreave F, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. Am J Respir Crit Care Med 2011; 184: 1125–32.

133. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo controlled trial. Lancet 2012; 380: 651–59.

134. Busse WW, Katial R, Gossage D, et al. Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor alpha antibody, in a phase I study of subjects with mild asthma. J Allergy Clin Immunol 2010; 125: 1237–44.

135. Wills-Karp M, Luyimbazi J, Xu X, et al. Interleukin-13: central mediator of allergic asthma. Science 1998; 282: 2258–61.

136. Piper E, Brightling C, Niven R, et al. A phase II placebo-controlled study of tralokinumab in moderate-to-severe asthma. Eur Respir J 2013; 41: 330–38.

137. Humbert M, de Blay F, Garcia G, et al. Masitinib, a c-kit/PDGF receptor tyrosine kinase inhibitor, improves disease control in severe corticosteroid-dependent asthmatics. Allergy 2009; 64: 1194–01.

138. Busse WW, Israel E, Nelson HS, et al. Daclizumab improves asthma control in patients with moderate to severe persistent asthma: a randomized, controlled trial. Am J Respir Crit Care Med 2008; 178: 1002–08

139. Tong X et al Macrolide antibiotics for treatment of asthma in adults: a meta-analysis of 18 randomized controlled clinical studies. Pulm Pharmacol Ther 2015 Apr;31:99-108

140. Graham V, Lasserson T, Rowe BH. Antibiotics for acute asthma. Cochrane Database Syst Rev 2001 ;(3):CD002741.

141. Wayne C.H. Wanga, Kathryn A. Mihlbachlera, Eugene R. Bleeckerb, Scott T. Weissc, and Stephen B. Liggetta,A Polymorphism of GRK5 Alters Agonist-Promoted desensitization of β2-Adrenergic Receptors, Pharmacogenet Genomics. 2008 August; 18(8): 729–32.

142. Faiza Baameur et al, Role for the Regulator of G-Protein Signaling Homology Domain of G Protein-Coupled Receptor Kinases 5 and 6 in β2-Adrenergic Receptor and Rhodopsin Phosphorylation, Mol Pharmacol 2010 77:405–15

143. Walker et al. G protein coupled receptor kinase 5 regulates airway response induced by muscarinic receptor activation. A J Physiol Lung Cell Mol Physiol. 2004; 286:312-9.

144. V. H. J. van der Velden, Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma, Mediators of Inflammation, 1998, 7, 229–37

145. Judith C.W. Mak, Takeshi Hisada, Michael Salmon, Peter J. Barnes & K. Fan Chung, Glucocorticoids reverse IL-1b-induced impairment of β-adrenoceptor-mediated relaxation and up-regulation of G-protein-coupled receptor kinases, British Journal of Pharmacology, 2002;135, 987-996

146. James E. Gern & William W. Busse Relationship of viral infections to wheezing illnesses and asthma Nature Reviews Immunology 2, 132-138

147. Neumann, E. *et al.* (2014) G protein-coupled receptors in rheumatology *Nat Rev Rheumatol.* 429–436

148. Eshan Ghosh et al Methodological advances: the unsung heroes of the GPCR structural revolution Nature Reviews Molecular Cell Biology 16, 69–81(2015)

149. Global strategy for asthma management and prevention.(2014) http://www.ginasthma.org, Page 32

150. Peter M. Jehle Steroid-induced osteoporosis: how can it be avoided?, Nephrol Dial Transplant (2003) 18: 861–864

151.A.N. Aggarwal, K. Chaudhry, S.K. Chhabra, G.A. D'Souza, D. Gupta, S.K. Jindal. Prevalence and Risk Factors for Bronchial Asthma in Indian Adults: A Multicentre Study. Indian J Chest Dis Allied Sci 2006; 48: 13-22

152. Liggett SB, Cresci S, Kelly RJ, et al. A GRK5 polymorphism that inhibitsbeta-adrenergic receptor signaling is protective in heart failure. Nat Med.2008; 14:510-517.

153. J. Sunyer, J.M. Antó, M. Kogevinas et al. Risk factors for asthma in young adults .Eur Respir J 1997; 10: 2490–2494

154. Jindal et al. Prevalence and Risk Factors for Bronchial Asthma in Indian Adults: A Multicentre Study. Indian J Chest Dis Allied Sci 2006; 48: 13-22

155. Delgado P Barranco S Quirce Obesity and Asthma J Investig Allergol Clin Immunol 2008; Vol. 18(6): 420-425

156. Roland Leung, Philip Ho,Asthma, allergy, and atopy in three south-east Asian populations Thorax 1994;49:1205-1210.

157.Wylie Burke, MD, PhD, Megan Fesinmeyer, Kate Reed, MPH, Lindsay Hampson, Chris Carlsten, MD Family History as a Predictor of Asthma RiskAm J Prev Med 2003;24(2) 160-169

158. Mahram et alThe Frequency of Common Allergens in AllergicRhinitis among the Patients Referred to the Allergy Clinic of Qods Hospital in Qazvin during 2007-2010,J Aller Ther Volume 4 • Issue 1 1-5

159. Te-Chun Shen et al Risk of Obstructive Sleep Apnea in AdultPatients with Asthma: A Population-BasedCohort Study in Taiwan PLOS ONE | DOI:10.1371, 1-11

160.Toni O. Kiljander, MD, PhD; and Jukka O. Laitinen, MD, PhD The Prevalence of Gastroesophageal Reflux Disease in Adult Asthmatics CHEST / 126 / 5 / NOVEMBER, 2004 1490-1494

161. Yıldız F, On behalf of the ASIT Study Group. Factors influencing asthma control: results of a real-life prospective observational asthma inhaler treatment (ASIT) study. Journal of Asthma and Allergy. 2013; 6:93-101.

162. Nathan RA, Sorkness CA, Kosinski M et al. Development of the asthmacontrol test: a survey for assessing asthma control. J.AllergyClin. Immunol.2004; **113**: 59–65.

163.Bisaccioni C, Aun MV, Cajuela E, Kalil J, Agondi RC, Giavina-Bianchi P. Comorbidities in Severe Asthma: Frequency of Rhinitis, Nasal Polyposis, Gastroesophageal Reflux Disease, Vocal Cord Dysfunction and Bronchiectasis. Clinics (Sao Paulo, Brazil). 2009; 64(8):769-773

164. L-P. Boulet Influence of comorbid conditions on asthmaEurRespir J 2009; 33: 897–906

# *Annexures*

# Genomic DNA gel electrophoresis

## Image.1: Genomic DNA Agarose gel electrophoresis

Image shows 0.8% agarose gel electrophoresis of the genomic DNA. Lanes shows the extracted DNA under UV trans illumination on ethidium bromide staining

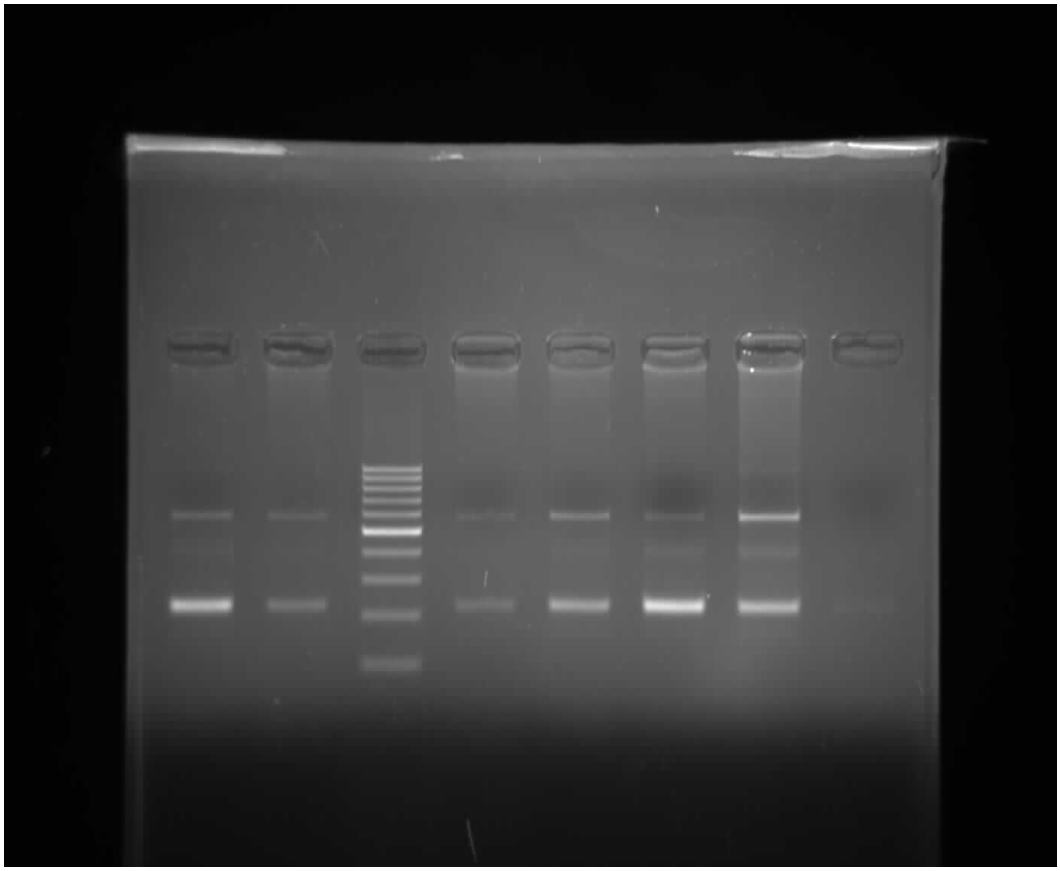


**Image.2: 15% Polyacrylamide gel electrophoresis of restrict digestion product**

Lane1 shows the DNA ladder.Lanes 6,10 shows a heterozygous
Rest shows wild type homozygous product.

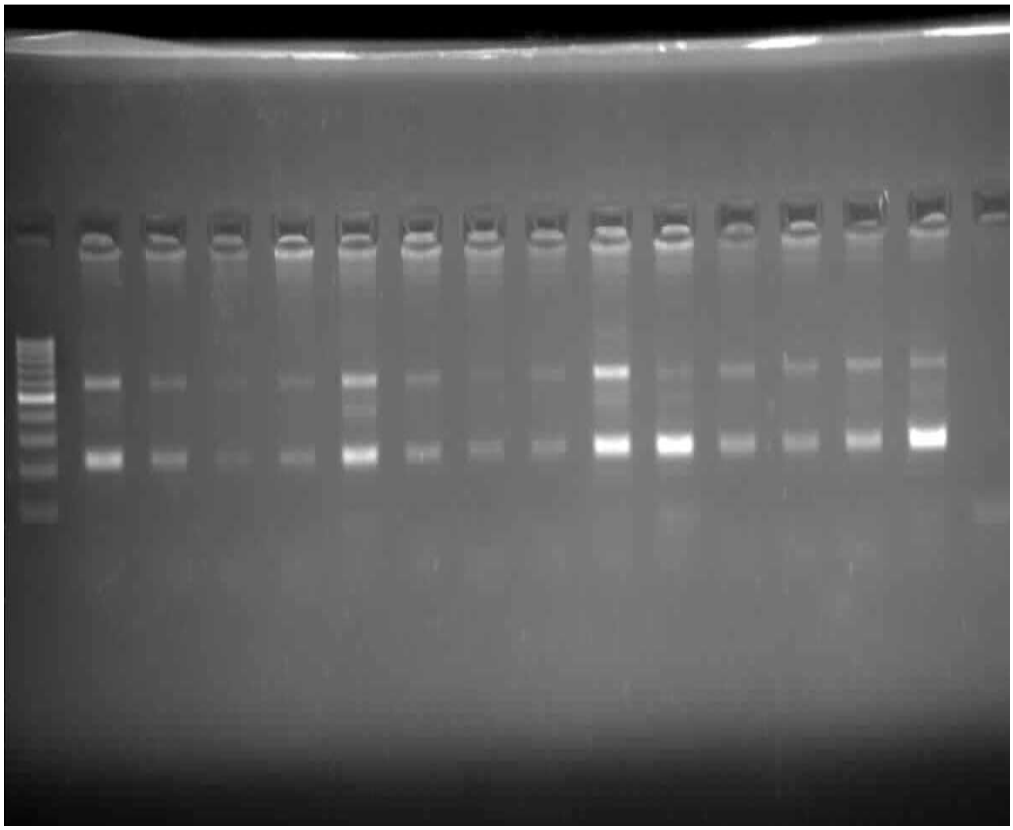
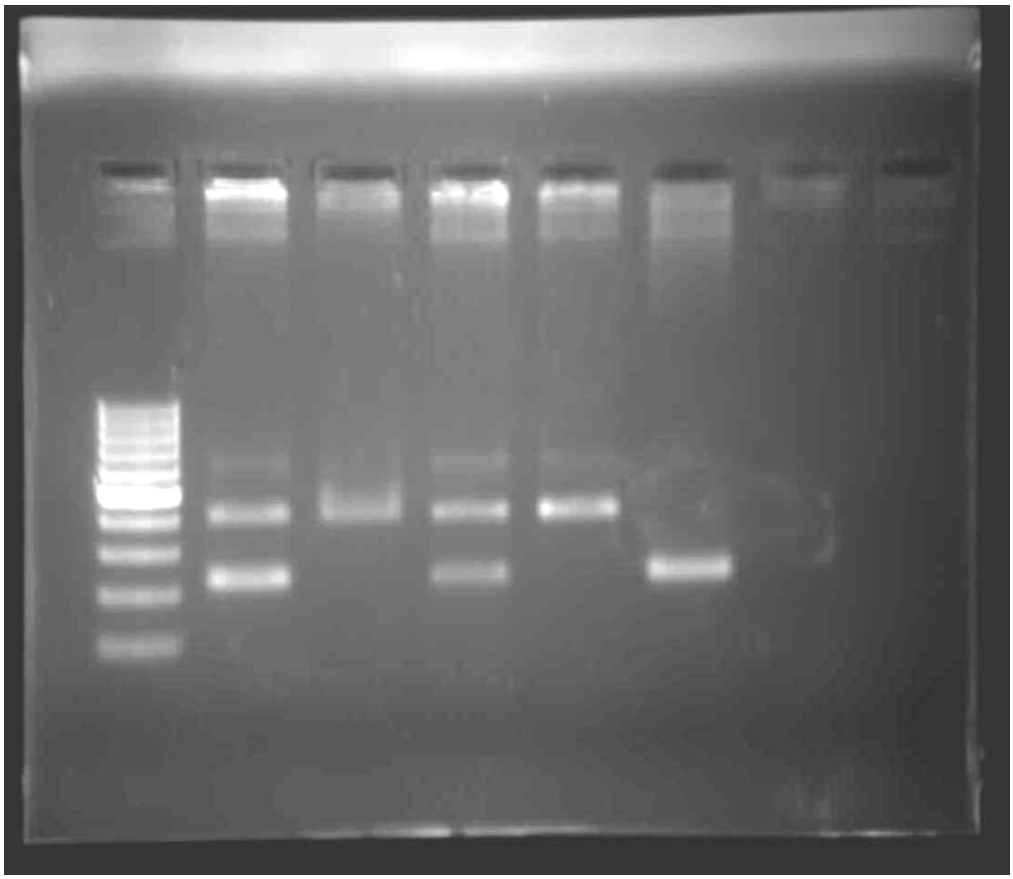


**Image.3: Genomic DNA Agarose gel electrophoresis**

Image shows 0.8% agarose gel electrophoresis of the genomic DNA.Lanes shows the extracted DNA under UV transillumination on ethidium bromide staining

Lane 1 shows ladder 2,4 shows heterozygous . Lane 3,5 shows mutant homozygous



**A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma**

**Dr.E.AmudhanArvind, Dr.S.Ramalingam**

## Case Report Form

Patient name: IP/OP no:

Age/Gender: Address:

Contact number:

Height: Weight:
BMI;

Occupation– (Current &Past):

Any Hazardous exposure in work place:

Ethnicity (Religion/caste)

H/o known allergens:

### Personal History:

Smoking – (cigar/beedi, how many, how long)

Alcohol-

Other Substance abuse-

### Family History

(H/O, asthma, atopic dermatitis, Eczema, allergic rhinitis)

### Disease related details

Asthma-> Duration of disease
Drug
Dose
Duration
Started taking from:
Route:
Change in drug in recent past:
Reason for change of drug:

2) Co morbid conditions & treatment:

Respiratory system related:

Non Respiratory system related:

HT-

DM-

TB-

Others-

H/O Obstructive Sleep Apnea

3) No of emergency visits in last year-

4) No of hospitalization in last year-

5) Treatment given:

5) During past 4 weeks have you had any

-respiratory infection

-GERD symptoms

-Rhino sinusitis

Pet/farm animals in house:

Other relevant history:

**Genotypic status of the patient**:

# PSG Institute of Medical Sciences & Research
## Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in


June 12, 2015

To
Dr E Amudhan Arvind
Postgraduate
Department of Pharmacology
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 12th June, 2015 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your application to renew the study entitled:

*"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma"*

The following documents were received for review:

1. Application for renewal dated 01.06.2015

After due consideration, the Committee has decided to renew the approval for the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

| Name | Qualification | Responsibility in IHEC | Gender | Affiliation to the Institution Yes/No | Present at the meeting Yes/No |
|---|---|---|---|---|---|
| Dr P Sathyan | DO, DNB | Clinician, Chairperson | Male | No | Yes |
| Dr S Bhuvaneshwari | M.D | Clinical Pharmacologist Member – Secretary | Female | Yes | Yes |
| Dr S Shanthakumari | MD | Pathology, Ethicist | Female | Yes | Yes |
| Dr Sudha Ramalingam | MD | Epidemiologist Alt. Member - Secretary | Female | Yes | Yes |
| Dr D Vijaya | M Sc., Ph D | Basic Medical Sciences (Biochemistry) | Female | Yes | Yes |

The renewal is valid for one year (From 16.06.2015 to 15.06.2016).

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
COIMBATORE-641004
INSTITUTIONAL HUMAN ETHICS COMMITTEE

**Dr S Bhuvaneshwari**
**Member - Secretary**
**Institutional Human Ethics Committee**

# PSG Institute of Medical Sciences & Research
## Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in


June 19, 2015

To
Dr E Amudhan Arvind
Postgraduate
Department of Pharmacology
PSG IMS & R
Coimbatore

The Institutional Human Ethics Committee, PSG IMS & R, Coimbatore -4, has reviewed your proposal on 19th June, 2015 in its expedited review meeting held at IHEC Secretariat, PSG IMS&R, between 10.00 am and 11.00 am, and discussed your request to include leftover samples in the study entitled:

*"A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma"*

The following documents were received for review:

1. Your letter dated 17.06.2015
2. Amendment reporting form
3. Informed consent forms (Ver 1.1)

After due consideration, the Committee has decided to approve your request to include leftover samples in the above study.

The members who attended the meeting held on at which your proposal was discussed, are listed below:

| Name | Qualification | Responsibility in IHEC | Gender | Affiliation to the Institution Yes/No | Present at the meeting Yes/No |
|---|---|---|---|---|---|
| Dr P Sathyan | DO, DNB | Clinician, Chairperson | Male | No | Yes |
| Dr S Bhuvaneshwari | M.D | Clinical Pharmacologist Member – Secretary | Female | Yes | Yes |
| Dr S Shanthakumari | MD | Pathology, Ethicist | Female | Yes | Yes |
| Dr Sudha Ramalingam | MD | Epidemiologist Alt. Member - Secretary | Female | Yes | Yes |
| Dr D Vijaya | M Sc., Ph D | Basic Medical Sciences (Biochemistry) | Female | Yes | Yes |

This Ethics Committee is organized and operates according to Good Clinical Practice and Schedule Y requirements.

Non-adherence to the Standard Operating Procedures (SOP) of the Institutional Human Ethics Committee (IHEC) and national and international ethical guidelines shall result in withdrawal of approval (suspension or termination of the study). SOP will be revised from time to time and revisions are applicable prospectively to ongoing studies approved prior to such revisions.

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Yours truly,

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

**Dr S Bhuvaneshwari**
**Member - Secretary**
**Institutional Human Ethics Committee**

***Proposal No. 14/160***

Patient's Name:_____ Today's Date:_____

# Asthma Control Test™ (ACT) is:

- A quick test that provides a numerical score to assess asthma control.
- Recognized by the National Institutes of Health (NIH) in its 2007 asthma guidelines.[1]
- Clinically validated against spirometry and specialist assessment.[2]

## PATIENTS:

1. Answer each question and write the answer number in the box to the right of each question.
2. Add your answers and write your total score in the TOTAL box shown below.
3. Discuss your results with your doctor.

| | | | | | | SCORE |
|---|---|---|---|---|---|---|
| **1.** In the past **4 weeks**, how much of the time did your **asthma** keep you from getting as much done at work, school or at home? | All of the time 1 | Most of the time 2 | Some of the time 3 | A little of the time 4 | None of the time 5 | |
| **2.** During the past **4 weeks**, how often have you had shortness of breath? | More than once a day 1 | Once a day 2 | 3 to 6 times a week 3 | Once or twice a week 4 | Not at all 5 | |
| **3.** During the past **4 weeks**, how often did your **asthma** symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning? | 4 or more nights a week 1 | 2 or 3 nights a week 2 | Once a week 3 | Once or twice 4 | Not at all 5 | |
| **4.** During the past **4 weeks**, how often have you used your rescue inhaler or nebulizer medication (such as albuterol)? | 3 or more times per day 1 | 1 or 2 times per day 2 | 2 or 3 times per week 3 | Once a week or less 4 | Not at all 5 | |
| **5.** How would you rate your **asthma** control during the **past 4 weeks**? | Not controlled at all 1 | Poorly controlled 2 | Somewhat controlled 3 | Well controlled 4 | Completely controlled 5 | |
| | | | | | | TOTAL |

## If your score is 19 or less, your asthma may not be under control.


The Asthma Control Test is for people with asthma 12 years and older.

## HEALTHCARE PROVIDER:

- Include the ACT score in your patient's chart to track asthma control.

**References: 1.** US Department of Health and Human Services, National Institutes of Health, National Heart, Lung and Blood Institute. *Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma (EPR-3 2007).* NIH Item No. 08-4051. http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm. Accessed September 10, 2007. **2.** Nathan RA et al. *J Allergy Clin Immunol.* 2004;113:59-65.

# ஆஸ்துமா கட்டுப்பாடு பரிசோதனை

**1.கடந்த 4 வாரங்களில், ஆஸ்துமாவினால் தாங்கள் , அலுவலகம், பள்ளி அல்லது வீட்டில் எவ்வளவு நேரம் வேலை செய்ய இயலாமல் போனது ?**

1 அனைத்து நேரமும்

.2. பெரும்பாலான நேரம்

3. சில நேரம்

4. மிக சிறிய நேரம்

5. முற்றிலும் இல்லை

**2. கடந்த 4 வாரங்களின் போது, எத்தனை முறை மூச்சுத்திணறல் இருந்தது?**

1. ஒரு நாளில் ஒரு முறைக்கு மேல்

2. ஒரு நாளில் ஒரு முறை

3. ஒரு வாரத்தில் 3 முதல் 6 முறை

4. ஒரு வாரத்தில் 1 அல்லது 2 முறை

5. முற்றிலும் இல்லை

**3. கடந்த 4 வாரங்களின் போது , எத்தனை முறை ஆஸ்த்மாவின் அறிகுறிகள் ஆன முச்சிதிணறல் இருமல், நெஞ்சு இறுக்கம் அல்லது வலி , இவற்றின் காரணமாக இரவு அல்லது விடியற் காலையில் வழக்கத்திற்கு மாறாக நீங்கள் படுக்கையில் இருந்து எழுந்து கொள்ள நேரிட்டது ?**

1. ஒரு வாரத்தில் 4 அல்லது அதற்கு மேற்பட்ட இரவுகளில்
2. ஒரு வாரத்தில் 2 அல்லது 3 இரவுகள்
3. வாரம் ஒரு முறை
4. ஒருமுறை அல்லது இருமுறை 4 வாரங்களில்
5. முற்றிலும் இல்லை

**4. கடந்த 4 வாரங்களில் போது, நீங்கள் உங்கள் மீட்பு இன்ஹேலர் அல்லது nebulizer மருந்து (அதாவது செரோப்லோ அஸ்தலின் போன்ற) எத்தனை முறை பயன்படுத்தினீர்கள்?**

1. ஒரு நாளைக்கு 3 அல்லது அதற்கு மேற்பட்ட முறை
2. நாளொன்றுக்கு 1 அல்லது 2 முறை
3. வாரத்திற்கு 2 அல்லது 3 முறை
4. வாரத்திற்கு 1 முறை
5. முற்றிலும் இல்லை

**5. கடந்த 4 வாரங்களின் போது உங்கள் ஆஸ்த்மாவின் கட்டுபாட்டை நீங்கள் எப்படி மதிப்பிடுவீர்கள் ?**

1. முற்றிலும் கட்டுப்பாட்டில் இல்லை
2. மோசமான கட்டுப்பாடு
3. ஓரளவு கட்டுப்பாடு
4. நன்கு கட்டுப்பாடு
5. முழுமையான கட்டுப்பாடு

**Total score :**

**PSG Institute of Medical Science and Research, Coimbatore**
**Institutional Human Ethics Committee**
**INFORMED CONSENT FOR RESEARCH PROJECTS**

I am **Dr.E.Amudhan Arvind** carrying out a study on the topic: **A study on the effect of GRK5 Gln41Leu polymorphism on response to glucocorticoid therapy in bronchial asthma**

As part of my research project being carried out under the aegis of the Department of:
**Pharmacology**

My research guide is: **Prof.Dr.S.Ramalingam**

**The justification for this study** is:

There is chance of variation in requirement of glucocorticoids in control of asthma due to polymorphism in this gene, this genotyping will help in identifying the variation in requirement and will help in determining the dosage required for good control.

**The objectives of this study are:**

**Primary Objective:**

1. To study the effect of GRK5 polymorphism on usage of glucocorticoids

**Secondary Objectives**

1. To study the influence of polymorphism on dosage and duration of treatment.
2. To compare the effect of GRK5 polymorphism on control of asthma in patients on glucocorticoids

**Sample size: 150 patients**
**Study participants** are: Bronchial asthma patients above the age of 18 on glucocorticoids

**Location**: PSGIMSR, Coimbatore

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

**Initial interview:** 10 to 15 minutes.

Data collected will be stored for a period of fifteen years. We will / will not use the data as part of another study.

**Blood sample collection: 2 ml directly from patient or from left over samples in Pathology and Biochemistry labs, these collected samples will not be used for any other purposes**
No. of times it will be collected: **Once**

Whether blood sample collection is part of routine procedure or for research (study) purpose:
**Research purpose**

Specify **purpose**, discomfort likely to be felt and side effects, if any: **To do genetic analysis and no discomfort or side effects**

Whether blood sample collected will be stored after study period: **NO**
Case details and data will be stored for 5 yrs

Whether blood sample collected will be sold: **No**

Whether blood sample collected will be shared with persons from another institution:**No**

**Medication** given, if any, duration, side effects, purpose, benefits: **No medications**

**Benefits** from this study: this genotyping will help in identifying the variation in requirement and will help in determining the dosage required for good control.

**Risks** involved by participating in this study: **No risks**

How the **results** will be used: the results will be used for **further researches and publications**

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

**Consent:** The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date:
Contact no:9894740443

Witness:

# ஒப்புதல் படிவம்

இ.அமுதன் அர்விந்த் ஆகிய நான் பி.எஸ்.ஜி மருத்துவ கல்லூரியில் பார்மக்காலஜி(மருந்தியல்) துறையின் கீழ் குளுகோகார்டிகாய்ட்(glucocorticoids) மருந்துகள் எடுக்கும் ஆஸ்துமா நோயாளிகளின் ஜி.ஆர்.கே 5 (GRK5) பாலிமார்பிசம் உண்டாக்கும் மாற்றங்கள் பற்றிய ஆய்வு மேற்கொள்ள உள்ளேன்

**என் ஆய்வு வழிகாட்டி**:பேராசிரியர்.மரு.ச.ராமலிங்கம்

**ஆய்வு மேற்கொள்வதற்கான அடிப்படை:** மரபனு மாற்றம் காரணமாக குளுகோகார்டிகாய்ட் தேவை மாறுபட வாய்ப்பு உள்ளது,இந்த மாற்றங்களை கண்டறிவதன் மூலம் ஆஸ்துமா நோயாளிகளின் நல்ல நோய் கட்டுபாட்டை கொண்டு வர உதவும்

**ஆய்வின் நோக்கங்கள்**

1. ஜி.ஆர்.கே 5 (GRK 5) பாலிமார்பிசம் (மரபனு மாற்றம்) மற்றும் குளுகோகார்டிகாய்ட் இடையே உள்ள தொடர்பு கண்டறிவது
2. மரபனு மாற்றம் காரணமாக குளுகோகார்டிகாய்ட் அளவு மற்றும் சிகிச்சை காலம் ஆகியவற்றில் உள்ள வேறுபாடுகள் கண்டறிவது.
3. நல்ல மற்றும் மோசமான கட்டுப்பாடு உடைய ஆஸ்துமா நோயாளிகளின் ஜி.ஆர்.கே 5 (GRK5) பாலிமார்பிசம் மற்றும் குளுகோகார்டிகாய்ட் தேவைக்கும் உள்ள தொடர்பு

**ஆய்வு மேற்கொள்ளபடும் இடம்:** பி.எஸ்.ஜி மருத்துவ கல்லூரி மருத்துவமனை

**ஆய்வின் பலன்**:வரும் காலங்களில் என் ஆய்வினால் சிறந்த முறையில் ஆஸ்துமாவை கட்டுபடுத்த இயலும்.

இந்த ஆய்வில் கிடைக்கும் தகவல்கள் 5 வருடங்கள் பாதுகாக்கப்படும்,**இவை வேறு எந்த ஆய்விற்கும் பயன்படுத்த பட மாட்டாது**,எந்த நிலையிலும் உங்களை பற்றிய தகவல்கள் யாருக்கும் தெரிவிக்க பட மாட்டாது அவை ரகசியமாக வைக்கபடும்

இந்த ஆய்வில் பங்கேற்க்க ஒத்துகொள்வதால் உங்களுக்கு எந்த பாதிப்பும் இருக்காது.எந்த நிலையில் வேண்டுமானாலும் ஆய்வில் இருந்து விலகி கொள்ளும் உரிமை உங்களுக்கு உண்டு.ஆய்வில் இருந்து விலகி கொள்வதால் உங்களுக்கு அளிக்கபடும் சிகிச்சையில் எந்த வித மாற்றமும் இருக்காது.

**இந்த ஆராய்ச்சிக்காக உங்களிடம் சில கேள்விகள் கேட்கபடும்.2 மி.லி. இரத்த மாதிரிகள் ஆய்விற்காக எடுக்கப்படும் அல்லது நீங்கள் வேறு பரிசோதனைக்கு பெத்தாலஜி மற்றும் பயோகெமிஸ்டிரி ஆய்வகத்தில்கொடுத்த இரத்த மாதிரியில் இருந்து எடுக்கபடும்.**

மேலும் இந்த ஆய்வில் பங்கு கொள்வது உங்கள் சொந்த விருப்பம்.இதில் எந்த வித கட்டாயமும் இல்லை.நீங்கள் விருப்பப் பட்டால் இந்த ஆய்வின் முடிவுகள் உங்களுக்கு தெரிவிக்கபடும்

**ஆய்வுகுட்படுபவரின் ஒப்புதல்:** நான் இந்த ஆராய்ச்சியின் நோக்கம் மற்றும் அதன் பயன்பாட்டினை பற்றி தெளிவாகவும் விளக்கமாகவும் தெரியப்படுத்தப் பட்டுள்ளேன்,இந்த ஆராய்ச்சியில் பங்கு கொள்ளவும் ,இந்த ஆராய்ச்சியின் மருத்துவ ரீதியான குறிப்புகளை வரும் காலத்தில் உபயோகபடுத்தி கொள்ளவும் முழு மனதுடன் சம்மதிக்கிறேன்

ஆய்வுக்குட்படுபவரின் பெயர்/முகவரி

ஆய்வாளரின் கையொப்பம்/தேதி:

கையொப்பம்&தேதி:

ஆய்வாளரின் தொலைபேசி எண்:9894740443
மனித நெறிமுறை குழு எண்:0422 2570170 EXTN: 5818

# ABBREVATIONS

WHO - World Health Organization

COPD - chronic obstructive pulmonary disease

SNP - Single Nucleotide Polymorphism

GRK - G protein-coupled receptor kinase

GPCR - G protein-coupled receptors

BTS - British Thoracic Society

NHLBI - NIH Heart, Lung and Blood Institute

GINA - Global Initiative for Asthma

IgE - Immunoglubulin E

PG - prostaglandin

LOX - Lipoxygenase

ASM - Airway smooth muscle

TH 1 & 2 – T-Helper cells 1&2

AHR – Airway Hyperresponsiveness

NF-KB - nuclear factor – kappa beta

GATA-3 - Trans-acting T-cell-specific transcription factor

PEFR - Peak Expiratory Flow Rate

BA - Bronchial Asthma

GERD – Gastro Esophageal Reflux Disease

OSA – Obstructive Sleep Apnoea

## STATISTICS

### RELATION BETWEEN REQUIREMENT OF HIGHER DOSE AND OTHER FACTORS INCLUDING SNP

| FACTOR | ODDS RATIO | CONFIDENTIAL INTERVAL | | "P" VALUE | SIGNIFICANCE |
|---|---|---|---|---|---|
| | | MINIMUM | MAXIMUM | | |
| SNP | 26.133 | 6.880 | 99.270 | <0.005 | SIGNIFICANT |
| BMI | 0.535 | 0.222 | 1.288 | 0.159 | NOT SIGNIFICANT |
| SEX | 2.089 | 0.579 | 5.019 | 0.095 | NOT SIGNIFICANT |
| HISTORY OF ALLERGY | 0.319 | 0.145 | 0.702 | 0.004 | NOT SIGNIFICANT |
| RESPIRATORY CO MORBIDITIES | 0.522 | 0.144 | 1.890 | 0.315 | NOT SIGNIFICANT |
| ALLERGIC RHINITIS | 1.263 | 0.578 | 2.673 | 0.558 | NOT SIGNIFICANT |
| PREVIOUS H/O OF TB | 3.700 | 0.874 | 15.670 | 0.060 | NOT SIGNIFICANT |
| OSA | 0.260 | 0.033 | 2.076 | 0.174 | NOT SIGNIFICANT |
| GERD | 1.144 | 0.344 | 3.089 | 0.826 | NOT SIGNIFICANT |
| CONTROL | 1.038 | 0.473 | 2.280 | 0.925 | NOT SIGNIFICANT |

## MULTIVARIATE REGRESSION ANALYSIS

| DOSE/GENOTYPE | Sig. | Exp(B) | 95% Confidence Interval for Exp(B) | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| SEX | .619 | .247 | .054 | 2.260 |
| CONTROL | .064 | 3.436 | .067 | 1.078 |
| BMI | .105 | 2.630 | .813 | 9.005 |
| ALLERGIES | .025 | 4.993 | 1.154 | 8.942 |
| RESPRELATED | .083 | 3.012 | .827 | 23.004 |
| H/O ALLERGIC RHNITIS | .179 | 1.810 | .157 | 3.410 |
| TB | .043 | 4.106 | .023 | 9.939 |
| OSA | .281 | 1.164 | .363 | 2.846 |
| GERD | .780 | .078 | .184 | 3.555 |
| GENOTYPE | .000 | 18.684 | 9.304 | 76.596 |

**RELATION BETWEEN LONG TERM THERAPY AND OTHER FACTORS INCLUDING SNP**

| FACTOR/DURATION | ODDS RATIO | CONFIDENTIAL INTERVAL | | "P" VALUE | SIGNIFICANCE |
|---|---|---|---|---|---|
| | | MINIMUM | MAXIMUM | | |
| SNP | 1.662 | 0.553 | 4.989 | 0.362 | NOT SIGNIFICANT |
| BMI | 0.592 | 0.297 | 1.182 | 0.136 | NOT SIGNIFICANT |
| SEX | 1.442 | 0.729 | 2.883 | 0.292 | NOT SIGNIFICANT |
| HISTORY OF ALLERGY | 1.243 | 0.633 | 2.442 | 0.528 | NOT SIGNIFICANT |
| RESPIRATORY CO MORBIDITIES | 1.368 | 0.517 | 3.622 | 0.527 | NOT SIGNIFICANT |
| ALLERGIC RHINITIS | 1.099 | 0.555 | 2.174 | 0.787 | NOT SIGNIFICANT |
| PREVIOUS H/O OF TB | 4.892 | 0.586 | 40.833 | 0.107 | NOT SIGNIFICANT |
| OSA | 0.745 | 0.237 | 2.338 | 0.613 | NOT SIGNIFICANT |
| GERD | 0.257 | 0.084 | 0.783 | 0.012 | NOT SIGNIFICANT |
| CONTROL | 1.004 | 0.512 | 1.968 | 0.991 | NOT SIGNIFICANT |

**MULTIVARIATE REGRESSION ANALYSIS**

| FACTORS | Sig. | Exp(B) | 95% Confidence Interval for Exp(B) | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| SEX | .625 | .831 | .396 | 1.744 |
| BMI | .165 | 1.686 | .807 | 3.525 |
| ALLERGIES | .350 | .693 | .321 | 1.495 |
| RESPRELATED | .608 | .752 | .253 | 2.236 |
| HOALLERGICRHNITIS | .631 | .831 | .391 | 1.767 |
| TB | .130 | .177 | .019 | 1.663 |
| OSA | .575 | 1.418 | .418 | 4.815 |
| GERD | .101 | 4.683 | 1.432 | 15.315 |
| DOSE | .749 | 1.185 | .419 | 3.350 |
| CONTROL | .641 | .829 | .377 | 1.823 |
| GENOTYPE | .284 | 2.197 | .521 | 9.267 |

**RELATION BETWEEN CONTROL AND OTHER FACTORS INCLUDING SNP**

| FACTOR | ODDS RATIO | CONFIDENTIAL INTERVAL | | "P" VALUE | SIGNIFICANCE |
|---|---|---|---|---|---|
| | | MINIMUM | MAXIMUM | | |
| SNP & GOOD CONTROL | 6.283 | 1.936 | 20.388 | 0.001 | SIGNIFICANT |
| POOR CONTROL | | | | | |
| SNP | 0.159 | 0.049 | 0.516 | 0.001 | NOT SIGNIFICANT |
| SEX | 1.705 | 0.860 | 3.379 | 0.125 | NOT SIGNIFICANT |
| BMI | 0.824 | 0.412 | 1.649 | 0.584 | NOT SIGNIFICANT |
| HAZADOUS EXPOSURE | 0.883 | 0.267 | 2.927 | 0.839 | NOT SIGNIFICANT |
| SMOKING | 2.263 | 0.596 | 8.660 | 0.220 | NOT SIGNIFICANT |
| H/O ALLERGY | 0.553 | 0.274 | 1.115 | 0.096 | NOT SIGNIFICANT |
| FAMILY HISTORY | 1.674 | 0.647 | 4.327 | 0.284 | NOT SIGNIFICANT |
| RESP CO -MORBIDITIES | 3.101 | 0.988 | 9.739 | 0.044 | SIGNIFICANT |
| ALLERGIC RHINITIS | 0.649 | 0.329 | 1.280 | 0.211 | NOT SIGNIFICANT |
| PREVIOUS H/O OF TB | 1.976 | 0.385 | 10.143 | 0.406 | NOT SIGNIFICANT |
| OSA | 3.850 | 0.821 | 18.046 | 0.068 | NOT SIGNIFICANT |
| GERD | 1.309 | 0.424 | 4.043 | 0.639 | NOT SIGNIFICANT |
| PETS | 1.767 | 0.449 | 6.954 | 0.410 | NOT SIGNIFICANT |

## MULTIVARIATE REGRESSION ANALYSIS

| CONTROL/GENOTYPE | Sig. | Exp(B) | 95% Confidence Interval for Exp(B) | |
|---|---|---|---|---|
| | | | Lower Bound | Upper Bound |
| SEX | .016 | .361 | .158 | .826 |
| BMI | .679 | 1.181 | .538 | 2.593 |
| HAZADROUSEXPOSURE | .947 | .954 | .235 | 3.863 |
| ALLERGIES | .026 | 2.653 | 1.127 | 6.248 |
| SMOKING | .202 | .376 | .083 | 1.693 |
| FAMILYNHO | .368 | .600 | .197 | 1.827 |
| RESPRELATED | .113 | .348 | .095 | 1.282 |
| HOALLERGICRHNITIS | .764 | 1.131 | .506 | 2.528 |
| TB | .664 | .664 | .105 | 4.214 |
| OSA | .166 | .305 | .057 | 1.639 |
| GERD | .434 | .605 | .171 | 2.133 |
| PET | .607 | .682 | .159 | 2.927 |
| GENOTYPE | .001 | 10.733 | 3.026 | 18.400 |

# MASTER CHART

| BAGS | AGE | SEX | GENOTYPE | CONTROL | DOSE | DURATION |
|---|---|---|---|---|---|---|
| BAGS 1 | **50** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 2 | **37** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 3 | **62** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 4 | **37** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 5 | **47** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 6 | **70** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 7 | **51** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 8 | **44** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 9 | **66** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 10 | **65** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 11 | **22** | **FEMALE** | **HETEROZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 12 | **46** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 13 | **49** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 14 | **60** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 15 | **59** | **FEMALE** | **HETEROZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 16 | **55** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 17 | **29** | **FEMALE** | **HETEROZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 18 | **47** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 19 | **45** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 20 | **53** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 21 | **58** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 22 | **54** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 23 | **46** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 24 | **32** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 25 | **53** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 26 | **50** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 27 | **75** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAGS 28 | **67** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 29 | **62** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 30 | **64** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 31 | **74** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 32 | **41** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 33 | **50** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 34 | **22** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 35 | **45** | **MALE** | **HETEROZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 36 | **42** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 37 | **59** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 38 | **57** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 39 | **62** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 40 | **57** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 41 | **18** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 42 | **48** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 43 | **70** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 44 | **81** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 45 | **85** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 46 | **45** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 47 | **58** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 48 | **22** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 49 | **50** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 50 | **53** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 51 | **55** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 52 | **21** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 53 | **73** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 54 | **75** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 55 | **63** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAGS 56 | **67** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 57 | **44** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 58 | **37** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 59 | **62** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 60 | **35** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 61 | **71** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 62 | **65** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 63 | **48** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 64 | **39** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 65 | **67** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 66 | **22** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 67 | **24** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 68 | **67** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 69 | **23** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 70 | **80** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 71 | **74** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 72 | **45** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 73 | **80** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 74 | **56** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 75 | **49** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 76 | **70** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 77 | **51** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 78 | **59** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 79 | **77** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 80 | **78** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 81 | **58** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 82 | **49** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 83 | **38** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAGS 84 | **26** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 85 | **51** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 86 | **67** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 87 | **19** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 88 | **53** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 89 | **67** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 90 | **46** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 91 | **33** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 92 | **45** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 93 | **59** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 94 | **22** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 95 | **43** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 96 | **50** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 97 | **37** | **FEMALE** | **WILD MUTANT** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 98 | **40** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **LONG TERM** |
| BAGS 99 | **68** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 100 | **34** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 101 | **22** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 102 | **61** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 103 | **41** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 104 | **49** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 105 | **49** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 106 | **50** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 107 | **41** | **FEMALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 108 | **69** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 109 | **37** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 110 | **70** | **FEMALE** | **WILD MUTANT** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 111 | **40** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAGS 112 | **62** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 113 | **67** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 114 | **63** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 115 | **42** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 116 | **75** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 117 | **45** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 118 | **33** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 119 | **37** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 120 | **75** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 121 | **43** | **FEMALE** | **WILD MUTANT** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 122 | **51** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **HIGH DOSE** | **SHORT TERM** |
| BAGS 123 | **38** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 124 | **65** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 125 | **21** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 126 | **39** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 127 | **36** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 128 | **59** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 129 | **44** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 130 | **67** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 131 | **56** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 132 | **31** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 133 | **67** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 134 | **43** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **SHORT TERM** |
| BAGS 135 | **54** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 136 | **87** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 137 | **47** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 138 | **52** | **MALE** | **HETEROZYGOUS** | **GOOD** | **HIGH DOSE** | **LONG TERM** |
| BAGS 139 | **43** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |

| | | | | | | |
|---|---|---|---|---|---|---|
| BAGS 140 | **26** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 141 | **63** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 142 | **26** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 143 | **34** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 144 | **58** | **MALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 145 | **40** | **FEMALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 146 | **46** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **SHORT TERM** |
| BAGS 147 | **18** | **MALE** | **WILD HOMOZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |
| BAGS 148 | **32** | **FEMALE** | **WILD HOMOZYGOUS** | **POOR** | **LOW DOSE** | **LONG TERM** |
| BAGS 149 | **49** | **MALE** | **HETEROZYGOUS** | **GOOD** | **LOW DOSE** | **LONG TERM** |